COMPARATIVE GENOMIC AND TRANSCRIPTOMIC ANALYSES OF *Xanthomonas citri* subsp. *citri* AND RELATED SPECIES PROVIDES INSIGHTS INTO VIRULENCE AND HOST-SPECIFICITY

By

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To my wonderful husband, Deepak, and our families, for their unconditional love and support in fulfilling my dreams

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TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CBC	Citrus bacterial canker
CBS	Citrus bacterial spot
CDS	Protein coding sequences
COG	Clusters of orthologous groups
CPS	Capsular polysaccharide
DDW	Double distilled water
EPS	Extracellular polysaccharide
HGT	Horizontal gene transfer
HR	Hypersensitive reaction/response
IS	Insertion sequence
LPS	Lipopolysaccharide
QS	Qorum sensing
Shared genes	Genes that are orthologous only in the strains compared in this study
TCS	Two component system
T1SS -T6SS	Type one secretion system - Type six secretion system
Unique/Singleton genes	Genes that are non-orthologous only in the strains compared in this study
Xacm	X*anthomonas axonopodis* pv. *citrumelo*
Xcaw	*Xanthomonas citri* subsp. *citri* strain A^w
Xcc	*Xanthomonas citri* subsp. *citri*
XccA	*Xanthomonas citri* subsp. *citri* strain A (306)
Xcv	*Xanthomonas campestris* pv. *vesicatoria* strain 85-10

Abstract of Dissertation Presented to the Graduate School
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Citrus canker caused by *Xanthomonas citri* subsp. *citri* (Xcc) has significant impact on citrus production worldwide. *X. axonopodis* pv. *citrumelo* (Xacm) is another citrus pathogen causing citrus bacterial spot disease which is geographically restricted within the state of Florida. Xcc is distinguished into different strains primarily by host range. The Asiatic strain (A) has a wide host range and is most virulent, whereas Wellington (A^w) strain has host range restricted to Mexican lime and alemow. We hypothesized that gene expression along with gene content, contributes to the difference in virulence and host range of closely related strains. We conducted comparative genomic analyses to study Xacm, A and A^w strains and transcriptomic analyses for A and A^w strains. Illumina, 454 sequencing and optical mapping were used to obtain complete genome sequences of Xacm strain F1 (4.9 Mb chromosome, no plasmid) and *X. citri* subsp. *citri* strain A^w12879 (Xcaw) (5.3 Mb chromosome and two plasmids pXcaw19 and pXcaw58). Comparative genomic analysis of Xacm to A strain showed differences in T3SS effectors, T4SS, LPS and others. In addition to *pthA*, putative effectors such as *xopE3*, *xopAI* and *hrpW* were absent in Xacm, which might

be responsible for reduced virulence of this pathogen compared to XccA. We also identified unique effectors like *xopC2* and *xopW* in Xacm that may be related to the restricted host range. Whole genome comparison of A^w to A strain, disclosed numerous genome rearrangements and insertion/deletion regions indicating genome plasticity. Protein blast revealed multiple unique genes in A^w including type III secretion system effectors *xopAF* and *xopAG*. Comparative genomic analysis showed various changes in genes related to LPS and T4SS. Furthermore, RNA-seq was used to compare expression profile of Xcaw and XccA strains in nutrient rich (NB) and plant intercellular space mimicking (XVM2) conditions using Illumina sequencing. Up-regulation of effector genes in Xcaw as compared to XccA might also contribute to its limited host range. The overexpression of genes involved in plant cell wall degradation, attachment, reactive oxygen species scavenging, nutrient transportation in XccA might contribute to its expanding of host range. Our data suggest that both gene content and gene expression contribute to difference in virulence and host-specificities of different strains.

CHAPTER 1
LITERATURE REVIEW

Introduction

Xanthomonas spp. belong to a very important genus of pathogenic bacteria causing various plant diseases (Ryan et al. 2011). These pathogens are Gram-negative rod shaped bacteria belonging to gamma-proteobacteria class and infect over 350 plant species (Chan and Goodwin 1999). *Xanthomonas* genus consists of 27 phytopathogen species most of them causing critical diseases to ornamental plants and crops (Ryan et al. 2011). The genus *Xanthomonas* affects 124 monocot and 268 dicot plant species, including nut and fruit trees, cereals, and brassicaceous and solanaceous plants (Bogdavone et al. 2011). Xanthomonads show characteristic uniformity in their physiological and morphological features. These unique characteristics within the genus present difficulties in establishing a stable phylogenic taxonomy reflective of both evolutionary inter-relationships and phenotypic diversity (Bogdavone et al. 2011). Vauterin et al. (1995) proposed a taxonomy that is the basis of current classification of these phytopathogens. The approach received further refinement by Rademaker et al. (2005) to increase its robustness. The closeness in phenotypic features is responsible for the convergent evolutionary pathogenic traits witnessed in *Xanthomonas* strains within similar species infecting same host(s) or different hosts differently (Rademaker et al. 2005).

Citrus Canker

Citrus canker is an important disease of most commercial citrus cultivars resulting in significant losses in Florida and other major citrus producing areas (Gottwald et al. 2001; Gottwald and Riley 2005). The disease is caused by

Xanthomonas citri subsp. *citri* (Xcc) (syn. *X. citri*, *X. axonopodis* pv. *citri, X. campestris* pv. *citri*) (Vauterin et al. 1995; Cubero and Graham 2002). Citrus canker disease is characterized by formation of raised circular, water soaked, necrotic lesions surrounded by a chlorotic halo on leaves, stems and fruits. On severely affected trees, citrus canker causes defoliation, twig dieback, general tree decline, blemished fruit and premature fruit drop. The disease cycle of Xcc is relatively simple where the bacteria colonize the plant apoplast, which eventually results in the degradation of the epidermal cells due to hyperplasia. Propagation of the bacteria occurs within lesions and it can take about 7-60 days for symptoms to appear (http://edis.ifas.ufl.edu/hs382). During wet weather when moisture flows freely into these lesions, the bacterial cells ooze out and are dispersed via windblown rain and enter the new plant hosts directly through stomata or through wounds, and grow in the intercellular spaces of the spongy mesophyll (Gottwald et al. 2002). Wind at speeds over 18 mph is enough to spread and aid the bacteria to penetrate plant stomatal openings or wounds inflicted on plants by insects, thorns, or pruning. The dispersed bacteria have limited lifespan under unfavorable conditions. Therefore, their survival in the natural environment is dependent on availability of a host and the ability to colonize it. For example, exposure to direct sunlight kills the bacteria, and those falling in the soil survive for few days or months. However, infected plant tissues are kept in dry conditions without exposure to sunlight and free from soil increases survival of the bacteria for years (Verniere et al. 2002).

Citrus canker is present in more than 30 citrus-producing countries in Asia, the Pacific and Indian Ocean islands, South America and the Southeastern United States (del Campo et al. 2009). The causal agent is considered a quarantine organism in

citrus-producing areas of Europe where canker has not been reported. Canker-free citrus-producing countries impose commercial restrictions on the transport and sale of citrus fruits from citrus canker infected areas. Canker greatly affects fresh citrus fruit, which comprises approximately 10% of Florida's $9 billion commercial citrus industry (FCM, 2012). The losses have major socioeconomic impacts in addition to hinderance to trees and losses in quality and quantity of fruit because of the perceptions of possible inoculum transmission on the fresh fruit product (Achor et al. 1996; Gottwald et al. 2001).

Management Strategies

Eradication was the major method of controlling citrus canker until it becomes endemic in most parts of Florida due to hurricanes. For eradication healthy trees in the radius of 1900 ft from infected trees were destroyed (Gottwald et al. 2002). Copper-based bactericides are currently the most effective management approach to control citrus canker especially in preventing infection of fruit. Successful management strategies in Florida still include the use of copper. However, copper resistance has been reported in Argentina (Canteros 2002). Although copper resistant strains have not yet been reported in Florida, there is potential for horizontal gene transfer of copper resistance genes from other closely and distantly related bacterial strains. Also, over time costs of using copper in the field have increased along with increasing copper resistance and environmental hazard concerns (Fu et al. 2012). The foreseeable long-term measures in containing citrus canker disease lies in application of biological based practices such as cultivation of disease resistant cultivars in regions where *Xcc* is endemic. Knowledge of virulence and host-range factors is the fundamental step in realizing success of this approach.

Host-Range Variation of Citrus Canker causing *Xanthomonas* spp.

Citrus canker is caused by the bacterial pathogen *Xanthomonas citri* subsp. *citri* (Xcc) and *Xanthomonas fuscans* subsp. *aurantifolii* (Xau). Based on the causal pathovar, host range and geographic distribution, Xau has been divided into two strains, B and C. *X. citri* subsp. *citri* induces Asiatic (A type) canker and is the most wide spread and virulent strain. Its origin has been linked to southeastern Asia, Indonesia or India (Civerolo 1984). It affects most *citrus* species with grapefruit (*C. paradisi*), Mexican lime (*C. aurantifolia*) and lemon (*C. limon*) being the most susceptible (Gabriel et al. 1988; Egel et al. 1991; Schubert et al. 2001). Within the A type canker, two variants type A^w and A*, have been described and are currently geographically limited to Florida and Southwest Asia, respectively (Verniere et al. 1998; Sun et al. 2004). The Xcc A* and A^W are phylogenetically most closely related to Xcc A strain, however both have restricted host ranges and cause disease on Mexican lime and few other varities.

Amongst the A type variants, the first to be isolated was Xcc variant (A*) a close relative of A strains. This variant was isolated in 1998 in south-west Asia and it mainly affects Mexican lime (Verniere et al. 1998). In 2003, researchers working in Southern Florida discovered another Xcc variant named (type A^W) (Sun et al. 2004). Type A^W bears similarities to A* but its host range is restricted to Alemow (*Citrus macrophylla)* in addition to Mexican lime. Therefore, strain A^W slightly differs from A* in terms of host range and disease phenotype. The A^w strain causes typical symptoms on Mexican lime but elicits a strong hypersensitive response (HR) in grapefruit. Xcc A* causes typical erumpent bacterial canker lesions on Mexican lime but shows reduced water-soaked and blister-like symptoms on grapefruit without causing HR (Das 2003). The other two canker types B and C are caused by two strains of *X. fuscans* subsp. *aurantifolii* that are

exclusively found in South America (Scubert et al. 2001; Das 2003). The B strain of citrus canker affects lemons in Argentina, Uruguay, and Paraguay. However, Mexican lime, sour orange, Rangpur lime, sweet lime, citron, and occasionally sweet orange and mandarin orange can also be affected. The C strain affects only Mexican lime growing in Brazil (Brunings and Gabriel, 2003).

X. axonopodis pv. *citrumelo* (Xacm) (syn *X. campestris* pv*. citrumelo*), a close relative of Xcc, causes Citrus Bacterial Spot disease (earlier known as Canker E) which is found only in Florida and is primarily restricted to nurseries (Graham and Gottwald 1990; Schoulties et al. 1987). This indigenous bacterium is restricted in infecting hybrid citrus, Swingle citrumelo and its trifoliate orange parent *Poncirus trifoliate,* under nurseries conditions, but shows very reduced bacterial populations in the grove (Stall and Civerolo 1991). Unlike strains of Xcc, *X. axonopodis* pv. *citrumelo* (Xacm) produces flat or sunken necrotic spots on leaf surfaces, with more prominent water soaked margins surrounding the necrotic areas, yellow halos on leaves and twigs (Stall and Civerolo 1991). These symptoms rarely appear on fruit and defoliation or dieback does not occur either (Stall and Civerolo 1991; Gottwald et al. 1991).

Significant progress has been made in understanding the infection and epidemiology of the citrus canker disease in the past decade (Burnings and Gabriel 2003; da Silva et al. 2002; Gottwald et al. 2001). The complete genome of XccA strain 306 was sequenced and compared with *X. campestris* pv. *campestris* (da Silva et al. 2002). It showed that the two bacteria share more than 80% of the genes and their chromosomal gene order is conserved. XccA has one circular chromosome consisting of 5,175,554 base pairs (bp), and two plasmids: pXAC33 (33,699 bp) and pXAC64

(64,920 bp). Genomic analyses showed that Xcc has an extensive repertoire of genes associated with pathogenicity and virulence which include effector encoding genes, genes coding cell wall degrading enzymes and secretion systems, genes for quorum sensing and Pathogen Associated Molecular Patterns (PAMPs) amongst others. It has been widely accepted that host range is not determined by attachment and the Type Three Secretion System (T3SS) but by the effector proteins that are delivered into plant cells. The effectors can be either *avr* (avirulence) or *pth* (pathogenicity) proteins and it is the effectors that result in limitation or extension of host range (Burnings and Gabriel 2003). Previous studies have reported virulence factors such as both *pthA* and *avr* proteins to be critical for the infection of citrus by Xac (Brunings and Gabriel 2003; Moreira et al. 2010).

It has previously been proposed that genetic makeup or genetic background of plant pathogen could influence the function of avirulence genes and the host specificity (Wang et al. 2006). A previous screening attempted to increase host range of Xcc A* strain by transferring the genomic library of Xcc A strain to Xcc A* strain by triparental mating. The conjugants were inoculated on grapefruit, which is not a host for Xcc A*. The study however did not result in any findings of host determinants (Al-Saadi 2005). In another screening the genomic library of *X. citri* subsp. *citri* strain A^w (Xcaw) was transferred to *X. perforans* and selected the transconjugants that can cause HR in grapefruit (Rybak et al. 2009). They found one avirulence gene, named *avrGf1*, which is present in Xcaw but not in Xcc A strain. The XcawΔ*avrGf1* strain causes less severe symptoms in grapefruit than a typical XccA strain (Rybak et al. 2009). Thus they suggest presence of other avirulence genes that may affect the host range and

virulence of Xcaw. The host range and pathogenicity can also be affected by other factors such as composition of lipopolysaccharide, as has been shown previously by Kingsley et al. where *Xacm* with mutation in *opsX* locus, encoding for LPS core assembly, lost its pathogenicity on citrus host plants but not on bean plants (Kingsley et al. 1993).

Virulence Related Mechanisms Used by Xcc

The finished genome of *X. citri* subsp*. citri* strain 306 (A) has been sequenced giving greater insights on how the pathogen uses key genes and genetic clusters in virulence and strategic invasion mechanisms (da Silva et al. 2002). Genes implicated in Xcc pathogenesis include those coding for bacterial surface structure and adhesion elements, toxins, type III secretion system (T3SS) and effectors, cell-wall degradation enzymes and *rpf* (regulation of pathogenicity factors) genes related to quorum sensing (da Silva et al. 2002). Attachment of the pathogen to the host is the initial critical step initiating the process of pathogenicity. The pathogen uses specialized surface structures to survive environmental stresses, attach the plant host and invade the plant intercellular space. These cell surface structures are encoded by extracellular polysaccharide (EPS), lipopolysaccharide (LPS), capsular polysaccharide (CPS), type IV pilli, adhesins and flagella genes.

EPS is secreted outside the cell and forms a layer on the outer surface. A cluster of 12 gum genes that produce xanthan gum encodes EPS production in Xanthomonas spp. Besides contributing to the bacterial survival against environmental stresses, EPS is a major component of biofilm of *Xanthomonas* spp. Mutations of *gum* genes in *Xanthomonas* spp. causes loss of EPS production, change in biofilm and impaired epiphytic survival on hosts (Chou et al. 1997; Dunger et al. 2007; Rigano et al. 2007;

Kim et al. 2009b). EPS is also important as virulence factor and has been shown to suppress callose deposition in the plant cell wall (Yun et al. 2006). CPS on the other hand, also forms a layer on the cell surface but unlike EPS is bound to the surface via a covalent bond. The capsule helps prevent cell desiccation, and aids in adherence to surface or other cells (Roberts 1996). Though the role of CPS in plant-pathogen interaction has not been throughly studied in *Xanthomonas*, mutation of genes like *opsX* and *galU* lead to the loss of capsule, changes in LPS and the loss of virulence suggesting that CPS is important for infection (Guo et al. 2010). The gene clusters of *Xanthomonas* spp. involved in LPS biosynthesis vary in number and sequence similarity (Lu et al. 2008). In Xcc, the two regions involved in LPS synthesis are one with genes encoding transferases, epimerases, and sugar transporters and another with genes encoding sugar biosynthesis (da Silva et al. 2002). LPS is a major outer membrane component of Gram-negative bacteria that contributes to the structural integrity of bacteria and protects it against the attack of toxic chemicals in environments. Mutations in LPS genes of Xcc leads to reduced biofilm formation, increased sensitivity to environmental stresses and reduction of virulence (Li and Wang 2011).

Type IV pili act as fimbrial adhesins helping the bacteria to adhere and colonize the plant host. The pili are filaments on cell surface that are responsible for bacterial twitching motility (Hirano and Upper 2000). Various genes including *fimA*, *fimT* and 26 *pil* genes encode type IV pilus biosynthesis in XccA (da Silva et al. 2002). Nonfimbrial adhesins that are type V secretion system substrates such as autotransporters and two-partner secretion substrates can also help in bacterial attachment to the host (Gerlach and Hensel 2007). Xcc contains multiple genes encoding nonfimbrial adhesins such as

xadA, *xadB* and filamentous hemagglutinins. Mutations in *fhaB* gene in Xcc abolished adhesion and biofilm formation and reduced virulence of Xcc, indicating that hemagglutinin proteins are important for tissue colonization (Gottig et al. 2009). Xcc has a full set of genes for flagellar biosynthesis and chemotaxis pathway (da Silva et al. 2002). Flagella are used not only for motility but also for surface attachment, biofilm formation and entry into the host (Josenhans and Suerbaum 2002). Mutations in *fliC* and *flgE* in Xcc, which encode flagellin and hook respectively, resulted in decreased motility and biofilm and also reduced virulence in host (Malamud et al. 2011).

The above mentioned bacterial surface structures like flagellin and LPS are important for virulence of the pathogens but also act as PAMPs, which can be recognized by plant surface-arrayed pattern recognition receptor-like kinases and induce PAMP- triggered immunity (Schneider and Collmer 2010). Major PAMP triggered basal defense responses in planta include oxidative burst, the production of reactive oxygen species (ROS), the production of antimicrobial compounds (phytoalexins), thickening of the plant cell wall, and expression of pathogenesis-related genes (Newman et al. 2007). Therefore, bacteria have to manipulate virulence traits for better growth in host and also suppress flagellar functions for avoidance of host defense response. Also, the bacteria use type three secretion systme to deliver 15-30 effectors directly into host cells to circumvent the PAMP triggered immunity and cause disease (Jones and Dangl 2006).

Plants in turn develop a more specialized mechanism to detect the effectors translocated by microbes, and activate a second layer of defense known as effector-triggered immunity (ETI) or also known as gene for gene resistance (Boller and He

2009). ETI involves the direct or indirect recognition of effector proteins by plant resistance (R) proteins. The R proteins are either nucleotide binding leucine rich repeat (NB-LRR) proteins or extracellular LRR proteins (Chisholm et al. 2006). ETI also induces oxidative burst, hormonal changes, and transcriptional reprogramming as common plant immune responses (Tsuda and Katagiri 2010). ETI is also associated with rapid plant cell death or hypersensitive reaction to restrict pathogen growth (Jones and Dangl 2006). ETI induces prolonged ROS production which acts as either signaling molecules or result in pathogen death. ETI also induces prolonged hormone signaling pathways specially one mediated by salicylic acid that is important for immunity of plants against bacteria (Tsuda and Katagiri 2010). Salycilic acid is the master regulator of the plant immune signaling network and thus suppresses microbial growth (Leon-Reyes et al. 2009). Not surprisingly, pathogens seem to have adapted effectors to overcome ETI by evading recognition and not by attacking ETI signaling (Tsuda and Katagiri 2010). Thus both the plant R genes and pathogen effectors are co-evolving in nature (Jones and Dangl 2006).

The secretion systems have been classified into six types in Gram-negative bacteria, type 1 to type 6, according to their composition, function and substrates (Tseng et al. 2009). All six secretion systems, are known to exist in Xcc (da Silva et al. 2002; Shrivastava and Mande 2008). Type I secretion system (T1SS) is a Sec-independent system that exports substrates in a one-step process across both membranes of bacteria. It consists of an ATP-binding cassette (ABC) transporter in the inner membrane, an outer membrane factor (OMF) serving as a protein channel and a membrane fusion protein (MFP) connecting the two components. T1SS plays an

important role in pathogenic bacteria by secreting toxins (e.g. hemolysins), lipases and proteases (Gerlach and Hensel 2007). Altough it has been known to be required for transport of an avirulence factor AvrXa21 which results in a host response in *X. oryzae* pv. *oryzae*, it has not been demonstrated to contribute to virulence in Xcc (da Silva et al. 2004).

Type II secretion system (T2SS) is a Sec-dependent system via which various potential virulence factors are secreted, including cell wall degrading enzymes (CWDEs), proteases, lipases and phosphatases. T2SS mediated translocation occurs in two steps: substrates with signal peptide are translocated across the inner membrane via the Sec pathway; and then they are exported across the outer membrane via the T2SS translocation pore, which is formed by approximately 12-15 components in the outer membrane (Sandkvist 2001). Xcc has two independent T2SS, which are encoded by *xcs* and *xps* gene clusters. Xcc also encodes for a large number of T2SS substrates most importantly the cell wall degrading enzymes like pectinolytic, cellulolitic and hemicellulolytic enzymes (da Silva et al. 2002).

Type III secretion system (T3SS) is a key pathogenicity factor conserved in plant and animal pathogenic bacteria such as *Yersinia* spp., *Shigella flexneri*, *Salmonella typhimurium*, *E. coli*, *Ervinia amylovora*, *P. syringae*, *Xanthomonas* spp. and others. It is a needle-like structure that delivers effector proteins directly from the bacterial cytoplasm into the host cells (Hueck 1998; Buttner and Bonas 2002). In plant pathogens, the T3SS genes are called *hrp* (hypersensitive response and pathogenicity) genes and *hrc* (hypersensitive response and conserved) genes. Those genes are required for bacterial pathogenicity and also for induction of hypersensitive response on

hosts and non-hosts, respectively (Lindgren et al. 1986; Alfano and Collmer 1997; Roine et al. 1997). T3SS effectors are secreted into host cells by T3SS. Many effector genes code avirulence factors that are recognized by specific plant resistance proteins, e.g., AvrBs1 in Xcv (Ronald and Staskawicz 1988). Several candidate effectors have also been identified based on homology to known effectors from other pathogens by *in silico* prediction (Noël et al. 2003). The major function of T3SS effector proteins is to optimize the host cell environment for bacterial growth either by interfering with host defense responses or by modifying the normal cellular function of host proteins (Nomura et al. 2005; Grant et al. 2006). This can be achieved by enzymatic activities of some T3SS effectors to modify host proteins and by transcription activator activities of effectors in AvrBs3/PthA family to alter host transcriptome. PthA effector identified in Xcc can confer ability to cause canker-like symptom to strains that do not cause canker symptoms like Xcam (Swarup et al. 1991). The effectors in AvrBs3/PthA family are transcription activators which target host transcription. PthA is the first member of AvrBs3/PthA family which was experimentally identified for its virulence activity (Swarup et al. 1991). It has been demonstrated that AvrBs3 acts as a transcription activator and binds to the promoter of *upa20*, which encodes a transcription factor that induces plant cell hypertrophy (Kay et al. 2007).

Type IV secretion system (T4SS), is a one-step secretion system that can transport macromolecules from bacterial cytoplasm into eukaryotic cells or other bacterial cells (Christie et al. 2005). The T4SS in *Agrobacterium tumefaciens* delivers T-DNA with protein from its Ti plasmid into the host to cause the formation of crown gall tumors (Christie et al. 2005). There are two T4SS clusters found in Xcc, one on the

chromosome and the other on plasmid pXAC64. However, neither cluster are complete as they lack of *virB5* and *virB7* in both clusters and the lack of *virD4* in the plasmid (da Silva et al. 2002). The products of those missing genes are important components for successful translocation of T4SS substrates. Hence, their influence on virulence remains unclear in Xcc (Yeo and Waksman 2004).

Type V secretion system (T5SS) is classified into three sub-groups based on the secretion mechanisms: T5aSS is the autotransporter system; T5bSS is the two-partner system and T5cSS is the oligomeric coiled-coil adhesin (Yen et al. 2002). T5aSS is an autotransporter containing three domains: a N-terminal signal peptide, a passenger domain and a translocation unit at C-terminal end. In contrast to the single polypeptide of T5aSS, T5bSS consists of two separate proteins (one passenger and one transporter) whereas T5cSS contains trimeric proteins for the formation of beta-barrel secondary structure. A large number of proteins, which are translocated via T5SS, contribute to bacterial virulence, including enzymes (proteases, peptidases, lipase, esterase), toxins, and adhesins (Gerlach and Hensel 2007).

Type VI secretion system (T6SS), was identified and characterized in *V. cholera* (Pukatzki et al. 2006) and *P. aeruginosa* (Mougous et al. 2006). Comparative genomic analysis revealed the presence of T6SS in more than 25% of sequenced bacterial genomes including Xcc (Shrivastava and Mande 2008). T6SS has been speculated to evolve from the bacteriophage base-plate, due to the homologies shared by several subunits of T6SS and subunits of the bacteriophage T4 tail spike (Cascales 2008). T6SS forms a phage-tail-spike-like complex to inject effector proteins directly into host cytoplasm like T3SS. It is required for virulence in animal and plant pathogenic bacteria

such as *V. cholera*, *P. aeruginosa*, *A. tumefaciens*, *X. oryza*e and others (Shrivastava and Mande 2008; Pukatzki et al. 2009)

Bacteria have evolved global regulatory networks to coordinate the expression of the large number of virulence traits discussed above. A few two-component signal transduction systems have been discovered to contribute to the global regulatory networks in *Xanthomonas* spp., including RavS/RavR, ColS/ColR, RpfC/RpfG, and response regulator HrpG. Two-component systems usually consist of a membrane-bond histidine kinase sensor and a cytoplasmic response regulator. On receiving an external signal, the histidine kinase sensor is autophosphorylated and subsequently transfers the phosphoryl group to the receiver domain response regulator. The activated response regulator then induces physiological changes by regulating the expression of target genes. A number of important physiological activities are under control of two-component systems in bacteria, including cell motility, biofilm formation, quorum sensing, and virulence.

Quorum sensing (QS) is a cell-to-cell communication method in response to fluctuation in cell-population. For QS, bacteria produce and release diffusible chemical signaling molecules into their environment. When the concentration of signaling molecules reaches a threshold, the bacteria detect and respond to this signal and alter their gene expression. In *Xanthomonas* two known signaling factors are diffusible signaling factor (DSF), which has been characterized as the unsaturated fatty acid cis-11-methyl-dodecenoic acid (Wang et al. 2004) and diffusible factor (DF), which is an uncharacterized butyrolactone molecule. DF controls the production of the yellow pigment xanthomonadin and EPS (Poplawsky and Chun 1997), whereas DSF-mediated

QS pathway regulates the production of extracellular enzymes (including proteases, pectinases and endoglucanase) and extracellular polysaccharides (EPS) as well as biofilm formation (Tang et al. 1991; Barber et al. 1997; Slater et al. 2000; Torres et al. 2007). The *rpf* gene cluster is responsible for DSF production and signal transduction, including the core genes *rpfF*, *rpfC* and *rpfG* (Chatterjee and Sonti 2002; He et al. 2006; Siciliano et al. 2006). The *rpfF* gene encodes a putative enoyl-CoA hydratase that catalyzes the synthesis of signal molecule DSF. Extracellular DSF is sensed by a two-component signal transduction system consisting of the sensor protein RpfC and response regulator RpfG. The downstream signaling pathway of QS in the bacterial cell is not completely understood. The demonstration that the HD-GYP domain of RpfG is a cyclic di-GMP phosphodiesterase indicates cyclic di-GMP is a second messenger in DSF signal transduction (Dow et al. 2006; Ryan et al. 2006). Cyclic di-GMP is synthesized by proteins containing GGDEF domain which has diguanylate cyclase activity, whereas cyclic di-GMP is degraded by proteins containing EAL or HD-GYP domains which have phosphodiesterase. The high levels of cyclic-di-GMP promote biofilm formation, while low levels promote motility and transcription of virulence factors (Simm et al. 2004; Tischler and Camilli 2004; Römling et al. 2005). One important target of cyclic-di-GMP is Clp (cAMP receptor protein–like protein) which is a transcriptional activator. Microarray analyses reveal that Clp is involved in the DSF-mediated QS system in *Xanthomonas spps.* (He et al. 2006; He et al. 2007; Guo et al. 2012). Cyclic-di-GMP binds to the Clp to prevent it from DNA binding and the induction of the expression of genes encoding extracellular enzymes, and genes involved in T3SS, and EPS biosynthesis (He et al. 2007). It is known that QS is required for full virulence of

Xcc in planta. A recent comprehensive study has shown that QS temporally regulates the expression of a large set of genes, including chemotaxis and flagellar biosynthesis, energy metabolism, T2SS substrates, T5SS adhesins, type IV pili, T3SS and T3SS effectors. The temporal regulation of QS regulon suggests that it is required at different stages of canker infection, including attachment, invasion and growth in host apoplast (Guo et al. 2012)

Other important regulator in *Xanthomonas* spp. consists of HrpG and HrpX that positively regulate the *hrp* gene cluster and various other cellular funtions (Wengelnik and Bonas 1996a; Wengelnik et al. 1996b; Guo et al. 2011). HrpG is a response regulator of OmpR family and works with an unknown sensor kinase to detect environmental signals. Significant induction of *hrpG* expression has been observed in minimal media (XVM2) or in plant apoplast, rather than in rich media or on leaf surface (Wengelnik et al.1996b). The activated HrpG positively controls the expression of *hrpX*, whose product is an AraC-type transcriptional activator. HrpX subsequently induces the expression of *hrp* gene cluster (Wengelnik and Bonas 1996a). HrpX binds to a conserved *cis*-regulatory element named plant inducible promoter, which is present in the promoter regions of *hrp* operons (Koebnik et al. 2006). A genome-wide microarray analysis in Xcc showed that HrpG and HrpX are global regulators in *Xanthomonas* spp. They control multiple cellular activities responding to the host environment, such as amino acid biosynthesis, oxidative phosphorylation, transport of sugar, iron and potassium, and others thus coordinating the infection of the pathogen (Guo et al. 2011).

Project Goals and Objectives

The present study aims to use comparative genomics and/or transcriptomics to understand the molecular mechanism responsible for difference in host range and

virulence in Xacm and Xcaw as compared to XccA. The hypothesis for the research is that differences in host-specificity and symptoms can be due to strain specific genes and also due to differences in gene expression/regulation. The goal this research was to identify critical genes involved in virulence and host-specificity in *X. citri* subsp. *citri* 306 and related strains.

The objectives were to (1) obtain complete genome sequence of *X. axonopodis* pv. *citrumelo* strain F1 and compare with other xanthomonads to understand and mine its genome for pathogenicity determinants for citrus bacterial spot disease and, (2) obtain complete genome sequence of Xcaw strain and compare its genome and transcriptome to XccA to investigate the mechanisms responsible for variation in host range and virulence. We applied a combination of techniques like 454-FLX sequencing, Illumina/Solexa sequencing and optical mapping to obtain high quality finished genome sequences of Xacm F1 and Xcaw12879. We then used RNA-Seq to compare the expression profile of XccA and Xcaw. High-throughput sequencing by Illumina was used to compare differences in transcriptomes of the two strains in nutrient rich and plant intrercellular space mimicking media XVM2.

CHAPTER 2
COMPARATIVE GENOMIC ANALYSIS OF *XANTHOMONAS axonopodis* pv*. citrumelo* F1 CAUSING CITRUS BACTERIAL SPOT AND RELATED STRAINS

Introduction

Xanthomonas is an important genus of plant pathogenic bacteria (Ryan et al. 2011). These Gram-negative rod shaped pathogens belong to class gamma-proteobacteria and can infect over 350 species of plants (Chan and Goodwin 1999). Among the diseases on citrus, citrus bacterial canker (CBC) and citrus bacterial spot (CBS) are caused by distinct pathovars of *Xanthomonas* species. Citrus canker is caused by several pathogenic variants of *Xanthomonas citri* (Xcc) (syn. *Xanthomonas campestris* pv*. citri* or *Xanthomonas axonopodis* pv. *citri*) (Schaad et al. 2006; Vauterin et al. 1995) whereas CBS is caused by *X. citri* pv*. citrumelo*. Xac strain 306 with a suspected origin in southeastern Asia causes Asiatic type (A) canker and is the most widespread and virulent form of CBC. It produces corky lesions as a results of hyperplasia and hypertrophy, surrounded by oily or water-soaked margins and a yellow halo on leaves, stems, and fruits.

In 1984, a disease similar to citrus canker was discovered in citrus nurseries in central Florida leading to destruction of millions of seedlings (Sun 1984). This new disease was mistakenly described as a form of citrus canker caused by “E” strain group also known as nursery strain canker. Leaf spots of this strain are irregular to round, 3–5 mm in diameter, flat, water-soaked, often necrotic in the center, and usually surrounded by a chlorotic halo. Water-soaked elongated lesions with necrotic centers are also observed on twigs but not on fruits (Cubero and Graham 2004). Extensive efforts were put forth to eradicate this disease, resulting in destruction of 20 million citrus plants at the cost of $94 million (Schubert 1991).

Strains in this group do not cause hyperplasia and the lesions continue to be flat with time unlike CBC, which results in raised callus-like lesions. Further research revealed that the CBS pathogen is variable but widely distributed in the state and does not have the same host range as Asiatic strain (Gottwald et al. 1993). CBS bacteria are most aggressive on trifoliate orange hybrids including Swingle citrumelo (Graham and Gottwald 1990). Populations of CBS bacteria developed to a lower level and varied in leaves of grapefruit (Schubert et al. 2001). The origin of the strain remains unknown as it is not found outside Florida, and is speculated to have moved to citrus from existing populations of *Xanthomonas* in Florida (Gottwald and Graham 1990). Further research showed that these strains are serologically, genetically and physiologically distinct from the previously known citrus canker pathogenic groups (Graham and Gottwald 1991) and are not susceptible to any of the phages commonly used to differentiate these groups (Graham et al. 1990). Because it affects citrus and causes symptoms that can be easily confused with canker, the disease was wrongly termed as E-strain citrus canker (Gottwald et al. 1988). The disease is now recognized as distinct from citrus canker and was named citrus bacterial spot caused by *Xanthomonas axonopodis* pv. *citrumelo* (Gabriel et al. 1989). Other names associated with the bacteria included *X. campestris* pv. *citrumelo* and *X. alfalfae* pv. *citrumelonis* (Gottwald et al. 1991; Schaad et al. 2006). The nomenclature and classification for the strains of *Xanthomonas* that infect citrus have undergone extensive taxonomic revision in recent years and are still under debate (Schaad et al. 2006; Vauterin et al. 2000). Hence, in this report we chose to use classical nomenclature and address the CBS pathogen as *Xanthomonas axonopodis*

pv. *citrumelo* (*Xacm*) as approved by Bergey's manual of systematic bacteriology (Saddler and Bradbury 2005).

Compared to XccA, Xacm has much reduced pathogenicity with limited host range. The host range of XccA is broad including most commercial citrus varieties while Xacm does not infect any commercial citrus varieties and it is limited primarily to trifoliate orange, its hybrids, and a few other individual species (Graham et al. 1990). Citrus bacterial spot occurs almost exclusively in nurseries, where young, susceptible tissue is abundant and irrigation is frequent (Timmer et al. 1991). In field, greenhouse, and growth chamber, Xacm generally does not cause disease when applied as a spray. Bacterial populations of bacterial spot strains within lesions on most hosts except Swingle citrumelo decline rapidly with time (Egel et al. 1991).

In comparison with XccA the Xacm strains isolated from Florida nurseries were found to vary widely in aggressiveness from each other (Graham and Gottwald 1990). Restriction Fragment Length Polymorphism analysis by Hartung and Civerolo (1989) showed that Xacm strains are not very closely related to XccA and they are not a form of canker. This was further corroborated by comparison of Xacm strains with other xanthomonads using DNA-DNA hybridization, which showed that Xacm is only about 60% similar to XccA (Egel et al. 1991). Later, Cubero and Graham deduced that Xacm is much closely related *to X. campestris* pv. *vesicatoria* rather than XccA based on 16S rDNA analysis (Cubero and Graham 2002) as well as leucine-responsive regulatory protein gene analysis (Cubero and Graham 2004).

The mechanism of reduced pathogenicity and limited host range of Xacm compared to XccA remains unknown. To address this question, comparative genomic

study was conducted in this present research by completing the genome sequence of Xacm strain F1 (Graham and Gottwald 1990). In comparison with XccA*, X. axonopodis pv. citrumelo* is a genetically, pathogenically, and serologically distinct pathogen (Alvarez et al. 1991; Gent et al. 2005; Graham and Gottwald 1990; Hartung and Civerolo 1989). We decided to sequence Xacm F1, which is a highly aggressive citrus bacterial spot bacterial strain, thus it is more likely to infect citrus nursery plants as a pathogen. To gain better understanding of ecological and evolutionary relationships between strains and species of *Xanthomonas*, we also compared Xacm with closely related strain of *X. campestris* pv. *vesicatoria* str. 85-10 (Xcv) (Syn. *X. euvesicatoria*; *X. axonopodis* pv. *vesicatoria*) causing bacterial spot on tomato and pepper (Lu et al. 2008; Ryan et al. 2011; Vauterin et al. 2000).

Materials and Methods

Bacterial Strain and DNA Sequencing

The *X. axonopodis* pv. *citrumelo* strain F1 sequenced in this study was isolated from Avon Park, Florida in 1984 and stored in a glycerol stock at -80°C. Genomic DNA was extracted from bacterial culture grown over night at 28°C in Nutrient broth medium, using a Wizard DNA purification kit (Promega, Madison, WI, USA.) according to the manufacturer's instructions. Quantity and purity of the DNA was measured spectrophotometrically (Nanodrop ND-1000, NanoDrop Tech. Inc., Wilmington, DE). Whole genome sequencing was performed using two high-throughput sequencing techniques, 454 pyrosequencing and Illumina Solexa GA sequencing. Single and paired-end reads were generated on a 454 GS-FLX Titanium sequencer (454 Life sciences, Branford, CT) in accordance with the manufacturer's protocol at Interdisciplinary Center for Biotechnology Research (ICBR), University of Florida.

Paired-end Illumina sequence reads were obtained using Illumina Genome Analyzer IIx (Illumina, Hayward, CA, USA) at Yale University Center for Genomics and Proteomics.

Gap Closure and Assembly Validation

The 1350 contigs obtained from Illumina were used to confirm the assembly of 454 scaffolds. The Illumina contigs were aligned against the 454 scaffolds using BLASTn to confirm the orientations and integrity of the assembled sequences and to close gaps and link contigs together within the scaffold. A *de novo* BamHI optical map of the genome of Xacm was generated by OpGen technologies (Madison, Wisconsin, USA). *In silico* BamHI restriction maps of the 5 scaffolds were constructed and aligned to the optical map according to their restriction fragment pattern, using MapSolver v.3.1 software (OpGen Technologies, Inc.). PCR primers were designed and Sanger sequences of these PCR products were used to close the gaps between the scaffolds. Final assembly was correlated with the optical map for further validation.

Annotation and Curation

Coding genes were identified using Softberry's FgenesB suite of bacterial operon and gene finding programs, based on Markov chain model prediction algorithm at ICBR, UF (Tyson et al. 2004). Predicted proteins were annotated by similarity searches against the NCBI Non-redundant (nr) protein database (http://ncbi.nlm.nih.gov) and clusters of orthologous groups (COG) database. A function was assigned to a predicted gene if it met the criteria of a minimum cutoff of 50% identity and 80% coverage of the gene length. In a few cases, additional putative protein-coding genes were annotated by direct homology search at the nr protein database using BLASTp. Each gene was also functionally classified by assigning a cluster of orthologous group (COG) number. The rRNA genes were annotated by the FgenesB tool based on sequence conservation,

while tRNA genes were detected with the tRNAscan-SE program (Lowe and Eddy 1997). Insertion sequences were identified by submitting the whole genome to the IS Finder website (Siguier et al. 2006). The CGView Server was used to generate graphical views of genome (Grant and Stothard 2008). The results of the automated annotation were examined and curated manually using the JGI GenePRIMP pipeline (Pati et al. 2010).

Phylogenetic Analysis

To determine the position of Xacm within the evolutionary tree of *Xanthomonas* pathovars, we used protein sequences of nine housekeeping genes *uvrD, secA, carA, recA, groEL, dnaK, atpD, gyrB* and *infB* from 10 completely sequenced *Xanthomonas* spps. We also added sequences from three *Xylella fastidiosa* strains and three *Pseudomonas* spp. as well as from two *Stenotrophomonas maltophilia* strains. The sequences of *Ralstonia solanacearum* strains GMI1000 & PSI07 and *Burkholderia cenocepacia* strain NCTC 10247 were used as out-group species. Amino acid sequences of nine proteins from the above genomes were aligned using clustal W (Larkin et al. 2007) and the resulting alignments were concatenated. Phylogenic tree from concatenated genes was constructed using PAUP 4.0 (Swarup et al. 1992) by the maximum likelihood method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches in the tree.

Comparative Analysis

For comparative analyses, the sequences of *X. campestris* pv. *vesicatoria* str. 85-10 (GenBank accession no. NC_007508) and *X*. *citri* subsp. *citri* str. 306 (GenBank accession no. NC_003919), which were determined as closest relatives to *X.*

campestris pv. *citrumelo* F1 in BLAST analyses as well as phylogenetic searches; were retrieved from GenBank. Complete genome sequences of all the three *Xanthomonas* spp. and also specific regions were aligned and visualized in progressive mode using MAUVE (Darling et al. 2010). Pan genome analysis that includes the core genome shared by all the three strains was done by an “all-against-all” BLAST of the protein sequences of the above genomes. The genes aligned based on amino acid sequence were considered orthologous if reciprocal BLASTp hits were found between two genes with e-value less than or equal to 10^{-20} and alignments exceeding 80% sequence identity and 80% query gene length. A gene was considered singleton to each strain if it had no hits with an *e*-values less than or equal to 10^{-5}.

Additional Sequence Analysis

Candidate T3SS effectors were identified using both nucleotide and protein blasts by comparison to the *Xanthomonas* effector database (http://www.xanthomonas.org). Putative perfect and imperfect PIP box sequences TTCGC-N_{15}-TTCGC and TTCGC-N_{16}-TTCG respectively were identified using custom scripts (Fenselau and Bonas 1995). Pathogenicity islands or genomic regions with atypical G+C content were identified using the web-based software GC-profile. It uses a suite of segmentation programs to identify regions with differential G+C content in the genome (Gao and Zhang 2006).

Pectate Lyase Assay

Cultures were grown on rich medium, nutrient agar at 28°C, then suspended in sterile deionized water and adjusted to the O.D. of 0.3 at 540nm. Hildebrand’s medium A, B and C which has pectate as the sole carbon source were used to test for pectolytic activity (Hildebrand 1971). The medium contained bromothymol blue dye, calcium

chloride, 2% sodium polypectae and 0.4% agar. The pH was adjusted to 4.5, 7.0 and 8.5 for the medium A, B and C. 1µl of the cultures were inoculated onto the plates and incubated at 28°C for 6 days before confirming pitting due to pectate lyase production.

Database Submission

The complete genome of *Xanthomonas axonopodis* pv. *citrumelo* strain F1 has been deposited at Genebank under the accession number CP002914.

Results

Sequencing and General Features of the Genome

Xacm was sequenced using 454 GS-FLX pyrosequencing (both unpaired and paired-end) (Margulies et al. 2005) and paired-end Illumina/Solexa sequencing (Bently et al. 2006). A total of 367,109 high-quality sequences with an average read length of 332 bp, representing more than 21-fold genome coverage were obtained by 454 FLX sequencing. These sequences were assembled into contigs and scaffolds using the 454 de-novo assembler Newbler 2.0 (Table 2-1). Although the genome coverage obtained through Illumina was much higher as compared to 454, the longer GS-FLX reads resulted in much better assembly of contigs. In total, 72 contigs were generated, of which 61 contigs were larger than 500 bp. The average size of the large contigs was 81 kb. These contigs were further grouped into five scaffolds based on paired-end reads. The maximum size of the scaffolds was 2,559,303 bases with an average of 990,948 bp. Solexa sequencing generated a total of 37,695,118 high-quality filtered sequence reads with an average read length of 74 bp. Average coverage was more than 400-fold. All reads were *de novo* assembled using CLCbio Genomics Workbench version 4.0, length fraction and similarity set at 0.9 and all the other parameters set as default values. This yielded 1,350 contigs (N_{50} = 8,322; maximum length = 36,202; minimum

length = 102). The 72 contigs obtained by 454 sequences were aligned in the right order to obtain 5 scaffolds using the paired-end reads. Aligning 1350 Illumina contigs and using the ones overlapping the 454-contigs solved most gaps within scaffolds. Pyrosequencing has a higher error rate around homopolymers (Huse et al. 2007) resulting in insertion-deletion errors in assembly and thus in-frame stop codons in genes. Illumina data on the other hand has errors mainly due to mismatches (Dohm et al. 2008). Hence, it was used to correct the errors in the scaffold sequences by mapping the Illumina reads against the 454 consensus sequences using CLCbio Genomics Workbench version 4.0 (Aury et al. 2008).

After all the intensive and time-consuming efforts the assembly still contained 5 scaffolds with internal gaps, which were difficult to resolve due to repeat regions. Thus optical mapping was used to obtain a *de novo* BamHI restriction map with no requirement for previous sequence information (Latreille et al. 2007). The *in-silico* restriction maps of scaffolds were aligned to this structural map to reveal the correct alignment and orientation of all the contigs as shown in Fig 2-1A. The genome was completely closed by primer walking and validated by manually inspecting all areas of imperfect match between the optical map and the sequence assembly (Fig 2-1B). The genome sequence was further corroborated by either high coverage with the 454 and Illumina data or by re-sequencing the region.

X. axonopodis pv. *citrumelo* strain F1 has a single, circular chromosome of 4,967,469 bp (Fig. 2-2) with no plasmids. Details of the general features of the genome are shown in Table 2-2. The G+C content of the chromosome averages 64.92%, which is similar to other *Xanthomonas* genomes. The chromosome displays a clear GC skew

transition typical of prokaryotic genomes, indicative of bi-directional replication mechanism (Ravin et al. 2003). GC skew analysis and blast comparison was used to locate the origin of replication at the point with an excess of G over C corresponding to the beginning of the leading strand and *dnaA* was first of the coding sequences (CDS) of the genome.

The Xacm genome encodes 4,202 putative coding sequences (CDSs), and 60 structural RNAs (Table 2-2). The genome shows a coding density of 86.53% characteristic of most xanthomonads. There is no asymmetry in the distribution of the CDS on the chromosome between the leading strand 2,131 (50%) and the lagging strand 2,131 (50%). After FgenesB annotation and manual curation, 3,481 CDSs (82.42%) could be assigned to one or more COG functional classes (Table 2-3) whereas there was not enough evidence for 721 CDSs to be assigned to any COG category. Two sets of 5S-16S-23S rRNA, clustered in operons were found located in a region of approximately 500 kb (between 4,379,256 bp and 4,847,563 bp) on the left replichore. A total of 54 tRNA genes with specificities for all 20 amino acids were also identified.

Phylogenetic Relatedness of Xacm to Other Xanthomonads

To establish the phylogenetic relationship of Xacm strain F1 with respect to other selected members of completely sequenced *Xanthomonas*, we compared a set of nine housekeeping genes (*uvrD, secA, carA, recA, groEL, dnaK, atpD, gyrB*, and *infB*). These genes are highly conserved that show no evidence of horizontal transfer among the 10 xanthomonads as well as other plant pathogens sequenced. These genes have provided robust analysis and resolved evolutionary relationships reliably in other studies (Clarke et al. 2010). For this analysis, we focused on bacteria with complete genomes

and excluded draft genomes due to the limitations of draft genomes (Palmer and McCombie 2002). We created an alignment of the nine proteins, concatenated the sequences and reconstructed the phylogenetic tree using maximum-likelihood method (Fig 2-3). The phylogenetic tree indicates that *X. axonopodis* pv. *citrumelo* strain F1 groups most closely with *X. campestris* pv. *vesicatoria* and *Xanthomonas citri* subsp. *citri*, forming a distinct clade from other xanthomonads. The closest relative of Xacm is Xcv 85-10 that causes bacterial spot disease in tomato and pepper (Thieme et al. 2005). This is consistent with the results by comparison of optical maps of the three chromosomes (Fig. 2-1C). Xacm and Xcv appear to be separated from XccA that is included in this cluster, which is supported by a good bootstrap value of 100 at this node. However, the relationship with XccA is sufficiently close that they share nucleotide sequence identity over 98% in most conserved regions. Interestingly, Xacm F1 has been shown to be 56% and 58% similar to XccA strain 9771 and Xcv strain 58, respectively by DNA-DNA hybridization analysis (Egel et al. 1991). It is noteworthy that other strains of XccA and Xcv were used in that comparison rather than the sequenced Xcc A strain 306 and *Xcv* strain 85-10. Interestingly, Gent and colleagues had shown that the pathovars of citrumelo are indistinguishable from a few other *X. axonopodis* pathovars and they do not form a monophyletic cluster by rep-PCR (Gent et al. 2005). However, Xacm F1 sequenced here is an aggressive strain which was not included in the previous study.

Comparison of Chromosome Organization of Xacm To XccA and Xcv

The chromosome organization of Xacm was compared with that of two closely related strains XccA and Xcv using MAUVE in progressive mode. Though most of the genome is collinear, Xacm harbors some translocations and inversions around the

replication terminus of the chromosome (Fig. 2-4). The unequal replichores might have been due from this reorganization. The Xacm genome has one major inversion with a translocation and 2 major deletions as compared to XccA, whereas there are 3 inversions with translocations and 2 major deletions compared to Xcv. Many of the rearranged and deleted blocks were flanked by transposons and/or integrases, indicating that this rearrangement may be a result of horizontal gene transfer.

The genome of Xacm does not harbor any plasmid. In comparison XccA contains two (pXAC33 and pXAC64) and Xcv contains four (pXCV2, pXCV19, pXCV38, pXCV183) plasmids respectively. Plasmids of xanthomonads have been reported to play important roles in pathogenicity. Plasmid pXAC64 of XccA encodes for *pthA4* gene, a homolog of *pthA*, which is capable of conferring ability to cause canker-like symptoms to strains of Xacm (Swofford 2003). On the other hand plasmids pXCV38 and pXCV183 encode for putative *Vir/Tra* and *Icm/Dot* like type IV secretion systems respectively (Thieme et al. 2005). The absence of plasmids from Xacm may have contributed to the reduced virulence of the CBS strain.

Horizontal Gene Transfer (HGT) and Genome Plasticity

Horizontal gene transfer is recognized as one of the major mechanisms for genome plasticity leading to diversification and speciation of the bacteria (Ochman et al. 2000). A simple method to identify potential horizontally transferred genes is to look for regions having atypical G+C content in the genome. The G+C content of Xacm genome ranged from 48.90% to 68.41% with an average of 64.92%. The segmentation results predicted eight regions of low GC content, which are recognized as genomic islands (Table 2-4). The negative cumulative GC profile of these regions is also different in

comparison to the whole genome (Fig. 2-5A). A sharp drop in the G+C content of these regions distinctly separates them from the rest of the genome (Fig. 2-5B).

These regions vary in size from approximately 3 kb to 64 kb. It was noteworthy that one of the genomic island from 2,970,113 bp to 3,004,362 bp encodes for *virB4*, *virB11*, *virB9* and *virD4* proteins that are part of type IV secretion system and components of type IV pilus like *fimT*, *pilE* and pilus tip-associated proteins. Presence of such genes in genomic regions indicative of horizontal gene transfer is in agreement with earlier reports (Thieme et al. 2005). It was also observed that about 50% of ORFs in the two biggest regions from 1,827,507 to 1,891,340 bp and 3,664,590 to 3,686,175 bp were determined to be orphan genes. Orphan genes have a very limited phylogenetic distribution and have no recognizable homologs. A recent study in *Escherichia coli* demonstrated that most orphan genes encode functional proteins (Daubin and Ochman 2004). Thus orphan genes may encode functional proteins in *Xacm* and might be responsible for virulence or differential host range of the strain.

In addition these regions have a high number of integrase and transposase genes, which is a conserved feature of genomic islands. The genome of *X. axonopodis* pv. *citrumelo* includes 41 insertion sequence (IS) elements. A majority of these transposases belong to IS3 family including Ixac2 and IS1404. We also found 2 IS elements from IS5 and IS1595 families each and 3 elements belonging to the Tn3 family.

Comparison of Proteins Encoded by Xacm to XccA and Xcv

We compared proteomes of the above three *Xanthomonas* spp. using reciprocal BLASTp. A Venn diagram representing the pan genome of all three genomes is shown in Fig. 2-6. The comparison of the predicted protein sequences revealed that 3,292

CDS are shared by all three genomes. These genes represent about three-quarters of the genome forming the core set that include conserved housekeeping and virulence genes essential for plant infection. Of the remaining 910 predicted genes in Xacm, 119 have homologs only in XccA and are absent from Xcv. These genes may include virulence factors necessary for infecting the common citrus host. The number of homologs in Xcv at 385 is much higher than XccA further confirming that the CBS strain is much closer to Xcv as compared to XccA. A total of 406 protein coding genes are unique to Xacm as compared to XccA and Xcv of which 298 are hypothetical proteins, 26 are mobile genetic elements and 82 are singletons with predicted functions. Moreover 174 genes show homologs in distinctly related *Xanthomonas* or other highly related bacteria suggesting their acquisition by horizontal gene transfer. The significant features shared between the genomes as well as the differences between the genomes are discussed in detail as follows.

Type Three Secretion Gene (T3SS) Clusters

Gram-negative bacteria use T3SS to translocate virulence factors into the host cell. In *X. axonopodis* pv. *citrumelo*, the T3SS is encoded by 27 genes, the organization of which is in close synteny with *hrp* cluster of Xcv and XccA (Fig. 2-7). The cluster includes all nine *hrc* (hypersensitive response conserved) genes that encode T3SS structural components and all 9 *hrp* genes some of which encode components of the *hrp* pilus. These genes, present only in phytopathogenic bacteria, are associated with rapid programmed death of plant cells at the site of infection in most non-host or resistant host plants and for pathogenesis in susceptible hosts (Buttner and Bonas 2003). The major difference as compared to XccA is that the Xacm cluster lacks the hypothetical protein upstream of *hrpF* and instead has three additional genes in the

same locus. These 3 genes consist of XACM_0383, which encodes a hypothetical protein with 97% amino acid identity to a hypothetical protein XPE_2921 in *X. perforans*, a pathogen of tomato causing bacterial spot (Jones et al. 1998); XACM_0384 that is 99% identical to outer membrane protein F1 (XopF1) from Xcv; and XACM_0385 with no obvious homologs. The final gene in this cluster, XACM_0407, shares 98% similarity to putative transglycosylase gene *hpaH* from *X. perforans*. As compared to Xcv, T3SS of Xacm lacks 2 *hrp* associated genes *hpaG* and *hpaE*, 2 outer protein genes *xopD* and *xopA* and 5 hypothetical genes XCV_0410, XCV_0412, XCV_0436, XCV_0438 and XCV_0439 with no known functions. Loss of these genes from Xacm might have contributed to host range and virulence variation.

Repertoire of T3SS Effectors of Xacm in Comparison to XccA and Xcv

T3SS effectors were identified in the Xacm genome and compared with XccA and Xcv. Considerable differences were observed in the effector repertoires present in these three strains (Table 2-5). Twenty-two effectors were identified in Xacm, whereas 25 and 30 effectors were identified in XccA and Xcv respectively. We subdivided them into three groups of core, partially shared and species-specific depending on their presence in the three strains. The core effectors shared by all the three strains consist of 17 effector genes. Of this core set, 9 effector genes (*avrBs2, xopK, xopL, xopN, xopP, xopQ, xopR, xopX, xopZ*) are present in genomes of all sequenced *Xanthomonas* with the exception of *X. albilineans* and *X. campestris* pv. *armoraciae,* the later of which has only *xopP* and *xopR*. These genes might be essential effector genes required for pathogenicity of xanthomonads in plant host. The effector *avrBs2* belongs to a family known for effector-triggered immunity in plants (Swords et al. 1996). It elicits HR in plants carrying *Bs2* resistance gene (Minsavage et al. 1990) and is needed for full

virulence of the pathogen on susceptible hosts. Effector *xopQ,* which belongs to the *hopQ1* family from *Pseudomonas* has also been known as an avirulence determinant in *Nicotiana benthamiana* since *Psedomonas syringae* pv. *tomato* DC3000 deletion mutant of *hopQ1-1* acquired the ability to grow to high levels and produce bacterial speck lesions in non-host *N. benthamiana* (Wei et al. 2007). XopN has been shown to interact with TARK1 and TFT1 proteins from tomato, thus repressing pathogen-associated molecular pattern triggered immunity (Kim et al. 2009). The homologs of effectors *xopL, xopP* and *xopQ* have been shown to contribute to pathogenicity in *X*. *campestris* pv. *campestris* (Jiang et al. 2009). Both *xopX* and *xopZ* potentially interfere with host innate immunity, thus making the plant more susceptible. The remaining 8 core effectors (*xopA, xopE1, xopF2, xopI, xopV, xopAD, xopAE, xopAK*) are not present in all xanthomonads and might be responsible for pathogenicity in some plant hosts while inducing resistance in others (White et al. 2009). It is likely that none of the effectors belonging to the core group are responsible for the difference in virulence and host range of Xacm, XccA, and Xcv.

The partially shared effectors are present in only two of the three strains. This group consists of *xopC1*, *xopF1* and *xopAJ* shared by Xacm and Xcv only as well as *xopE2* shared by XccA and Xcv but absent from *Xacm*. *xopF1* is found in all *Xanthomonas* species except *Xac* which encodes a truncated version of the same. In Xacm a homolog of *xopF1* is present which shares 99% similarity to that gene in Xcv. The *xopAJ* homolog of Xacm shares 99% similarity with *xopAJ*/*avrRxo1* of Xcv. This gene is truncated in Xacm due to a deletion mutation at 1056 bp in the gene that resulted in early termination of the protein at 379 amino acids as opposed to its

homolog of 450 amino acids in Xcv. The *xopC1* effector gene encodes a haloacid dehalogenase-like hydrolase with several phosphoribosyl transferase domains. This gene is present in Xacm but is fragmented across the genome (XACM_2129, XACM_2132 and XACM_2248) due to genome rearrangement and transposon insertion and thus is likely to be non-functional. *xopE2* which has been identified in various xanthomonads has recently been shown to be involved in virulence of Xcv group B strains on tomato but not in that of group A strains (Lin et al. 2011). It has also been related to suppression of HR indicating that it plays a dual role in different host plants (Lin et al. 2011).

Two species-specific effectors *xopC2* and *xopW* were found in Xacm. Though homologs of *xopC2* are found in both XccA and Xcv, they might be non-functional. Xacm consists of a *xopC2* gene with its closest homolog in *X. perforans* which causes bacterial spot only on tomato (Potnis et al. 2011). Xacm also has a truncated gene XACM_0435 which is a homolog of *xopW* from *X. oryzae* pv. *oryzicola* and might be non-functional. Xcv has at least 9 unique effectors as listed in Table 2-5. Some effectors like *avrBs1*, *avrBs1.1*, *xopJ3* are known avirulence factors. These effectors might be important for pathogenicity in tomato and pepper. XccA possesses four unique effectors *avrBs3/pthA, XopE3, XopAI* and *HrpW* which are absent in Xacm and Xcv (Table 2-5).

The differences in repertoire of T3SS effectors in Xacm and XccA might contribute to their difference in virulence and host range. T3SS effectors have been known to contribute to pathogenicity and multiplication of pathogens *in planta* (Gurlebeck et al. 2006). T3SS effectors benefit the pathogens by altering the

physiology of the host cell and suppressing plant defenses (Grant et al. 2006). T3SS effectors might contribute to host range by suppressing host defenses as virulence factors or narrowing the host range when certain effectors are specifically recognized by the plant as avirulence factors (Hajri et al. 2009). Importantly, *avrBs3/pthA* is present in XccA strain 306 while absent in Xacm F1 and Xcv 85-10. However, it is noteworthy that many other Xcv strains contain *avrBs3* and homologs (Szurek et al. 2002). In XccA strain 306, there are 4 copies of *pthA*: *pthA1*, *pthA2*, *pthA3* and *pthA4* on two plasmids, which are all absent in *Xacm* and *Xcv*. PthA4 with 17.5 repeats which is same as PthA is known to play an important role in citrus canker as knockout of *pthA4* abolished the development of citrus canker symptom development (Al-Saadi et al. 2007). PthA is responsible for development of hypertrophic and hyperplasic symptoms and cell death and its mutation leads to reduction in ability of bacteria to disseminate from infected lesions (Yang and White 2004). PthA also contributes to the epidermal rupture and necrosis, which promotes exudation and dissemination of XccA. Interestingly, the *pthA* gene from XccA strain when introduced to Xacm conferred the ability to cause raised pustules (Swarup et al. 1992). PthA and its homologs do not determine host-range according to a previous study (Al-Saadi 2005) indicating that neither of the complementing homologs nor any of the noncomplementing paralogs of *pthA* suppresses avirulence of Xcc A* strain on grapefruit. However, *hssB3*.0, a homolog of *pthA*, was shown to be responsible for host specific suppression of virulence of Xcc A strain KC21 on *Citrus grandis* cultivars but not on other *Citrus* species such as *Citrus sinensis* (Shiotani et al. 2007). This suppression led to reduced aggressiveness rather

than change in host range since Xcc A strain KC21 still causes citrus canker symptoms on *Citrus grandis*.

Other Xacm specific effectors might be contribute to the broader host range of XccA compared to Xacm. *xopE3* (*avrXacA2*) is a putative transglutaminase enzyme that belongs to the *hopX* (*avrPphE*) family and is widespread among phytopathogenic bacteria (Nimchuk et al. 2007). XopAI is putative effector protein reported only in the three canker causing strains XccA 306, *X. aurantifolii* strain B and *X. aurantifolii* strain C as well as in one *X. vesicatoria* str. 1111 (Moriera et al. 2010, Potnis et al. 2011). The role of XopAI in virulence of *Xanthomonas* remains to be characterized. HrpW is not known to be associated with virulence, although it contains domains resembling harpins and pectate lyases. It may not function as an intracellular effector but is secreted by the T3SS. HrpW in several other phytopathogens is known to elicit an HR in non-host plants (Kim and Beer 1998). Alternately, the limited host range of *Xacm* might result from the presence of the Xacm specific *xopC2* and *xopW* serving as avirulence factors. The presence of all the species-specific effectors in XccA and Xacm may be the main factors determining the host range of the pathogens. Further study is needed to understand their contribution to XccA and Xacm for infecting different hosts.

The difference in effector repertoires of Xacm and Xcv might contribute to their different host specificity with Xacm infecting citrus seedlings whereas Xcv 85-10 causing bacterial spot disease on both pepper and tomato plants (Potnis et al. 2011; Ryan et al. 2011). It has been suggested that the specific effector set of a given bacterial strain is the potential determinant of host range (Thieme et al. 2007). Compared to Xcv, Xacm contains *xopC2* and *xopW*, which are absent in Xcv while

Xacm lacks *avrBs1, xopB, xopD, xopG, xopH (avrBs1), xopJ1, xopJ3 (avrRxv), xopO, and xopAA* (Table 2-5), which are present in Xcv. *avrBs1* is known to encode a 50 kDa protein with homology to AvrA of *Pseudomonas syringae* pv*. glycinea*. This protein specifies avirulence on pepper cultivars containing the resistance gene *Bs1* (Napoli and Staskawicz 1987). XopD is known to alter host transcription, promote pathogen growth, and delay development of disease symptoms (Kim et al. 2008). XopB attenuated cell proliferation when expressed in yeast and also cause cell death in *N*. *benthamiana* leaves but not in tomato (Salomon et al. 2011). XopJ homologs are known to inhibit host protein secretion and interfere with defense responses (Bartetzko et al. 2009). Other effectors XopG, XopO, and XopAA have not been studied in detail in Xcv*.* How these effectors contribute to the different host ranges and different virulence of Xacm and Xcv needs further characterization.

Other Secretion Systems Associated with Virulence

Xanthomonads have at least five more protein secretion systems including type I to type VI other than the T3SS. Genes involved in all the secretion systems were identified in Xacm F1 genome. Secretion systems are of fundamental importance for translocation of proteins and other molecules. They play important roles in virulence of different bacterial pathogens. The relevant features of these virulence-associated secretion systems of Xacm shared with XccA and Xcv are presented below.

Type 1 Secretion System (T1SS)

T1SS has not been shown to contribute to virulence in *Xanthomonas* spp. (Buttner and Bonas 2010). Instead, T1SS is required for Xa21-mediated immunity in rice against *X. oryzae* pv*. oryzae* PXO99. The Ax21 (activator of Xa21-mediated immunity) is highly conserved in *Xanthomonas* spp. and secreted by T1SS. In Xacm the

Ax21 protein (XACM_0208) is 100% identical with XccA and Xcv proteins and 93% identical with *X. oryzae* pv. *oryzae* PXO99 protein. In addition, RaxST is required for sulfation and three genes *raxA*, *raxB* and *raxC* are required for secretion of Ax21 (Han et al. 2011). The gene *raxgA* in Xacm (XACM_1188) may be non-functional due to a frameshift mutation whereas the proteins encoded by *raxB* (XACM_1189), *raxC* (XACM_3355) and *raxST* (XACM_1187) are 99-100% identical to Xcv proteins. XccA on the other hand encodes for only *raxC* gene.

Type 2 Secretion System (T2SS)

Xacm is also equipped with the *xps* and *xcs* T2SS clusters, which secrete toxins and degradative enzymes. The T2SS clusters in Xacm are very conserved compared to those identified in Xcv and XccA with *xps* being composed of 11 genes and *xcs* of 12 genes (Fig 2-8). The xps T2SS that is found in all the sequenced xanthomonads is known to affect virulence in Xcv and also enhance translocation by T3SS. The *xcs* T2SS, on other hand is restricted to only some *Xanthomonas* spp. and has no obvious virulence function (Szczesny et al. 2010). The T2SS are known to secrete many plant cell wall degrading enzymes like cellulases, xylanases, lipases and proteases amongst others. Each species has its unique set of enzymes, which helps degrade components of the plant cell wall, thus assisting in pathogenesis. The range of these enzymes in Xacm was compared to the ones found in Xcv and XccA (Table 2-6). Enzymes such as cellulase, protease and pectate lyase have been known to promote bacterial nutrition and also virulence (Dow et al. 1990; Ray et al. 2000). XynC, an endoxyalanase in Xcv is known to be secreted by the *xps* T2SS under the control of *hrpG* and *hrpX* and contribute to virulence (Szczesny et al. 2010). Xacm contains a homolog of this gene, XACM_0913 that may play a similar role. Most of the enzymes show functional

redundancy and hence loss of one gene might not affect virulence. Rajeshwari *et al*. (Rajeshwari et al. 2005) showed that double mutants of both lipase and xylanase show much reduced virulence as compared to the single mutants in *X. oryzae* pv. *oryzae*.

It is interesting to note that Xacm is deficient in pectate lyase function. In comparison of the four genes in Xcv and three in XccA. Xacm shows presence of only two genes. However, both the genes XACM_2919 and XACM_3456 are pseudogenes that have stop codons resulting in truncated proteins. Thus, these proteins may be non-functional. This was confirmed by inoculating the strains onto Hildebrand's medium. Both XccA and Xcv produced pitting in the agar at pH 8.5 to confirm pectate lyase activity. Xacm did not produce any pitting as seen in Fig. 2-9, supporting the hypothesis that it is pectate lyase deficient. A pectate lyase homolog *xagP* has been shown to induce an HR in tobacco and pepper in *X. axonopodis* pv*. glycines* (Kaewnum et al. 2006). The role of pectate lyase in citrus pathogens remains to be determined. Several T2SS substrates have been shown to not only affect virulence but also induce plant defense responses. T2SS and its substrates are also controlled by T3SS regulators (Guo et al. 2011).

Type 4 Secretion System (T4SS)

In bacteria, the T4SS is known to contribute to virulence. Two T4SS clusters are present in both XccA and Xcv. Both the clusters are of Vir type in Xac (Fig. 2-10), where one is located on the chromosome and the other on plasmid (Al-Saadi et al. 2007). Xcv on the other hand encodes for one Vir and the other Dot/Icm type cluster (Fig. 2-10), both on plasmids and a partial Vir cluster on chromosome (Thieme et al. 2005). Xacm encodes for only one Vir type T4SS cluster on the chromosome. The cluster in Xacm does not show high similarity to Vir type T4SS of either XccA or Xcv. With the exception

of *virD4* that shows homologs in both XccA and Xcv, most of the predicted T4SS genes in Xacm share sequence similarity with genes in strains of *Stenotrophomonas maltophilia, which* is an aerobic gram-negative environmental bacterium commonly found in soil, water and animals (Hauben et al. 1999). Xacm also encodes for a *virK* and two *virJ* like proteins, outside the T4SS cluster. The function of *virK* protein is unknown and it has been linked to T2SS substrates instead of T4SS (Guo et al. 2011). VirJ is a periplasmic chaperone believed to mediate the association between the T4S pilus and substrate proteins (Christie et al. 2005).

Type 5 Secretion System (T5SS)

Both Xac and Xcv encode a two-partner secretion system, which belongs to T5SS. It translocates large proteins such as adhesins, and has been identified in many bacterial pathogens. Xacm encodes for a filamentous hemagglutinin like protein *fhaB*. A complete homolog of this gene can be found in XccA but is inactivated in Xcv by an internal stop codon inducing mutation. In Xacm, the gene is interrupted due to genomic rearrangements, which indicates that it might be inactive. The rearrangement has caused the gene to split with an insertion of 2 hypothetical proteins XACM_1838 and XACM_1839 between the two *fhaB* fragments. Transposon genes in the vicinity may have instigated this change. *fhaB* is involved in attachment and biofilm formation in XccA and its loss affects virulence of the bacterium (Gottig et al. 2009). The *fhaB* gene in Xacm is likely to be inactive due to the insertion and lack of the functional gene could contribute to the lower virulence of Xacm as compared to XccA.

Type 6 Secretion System (T6SS)

Recently a new secretion system was identified in *Vibrio cholerae* and *Pseudomonas aeruginosa named T6SS.* T6SS is evolutionarily related to

bacteriophage, likely a reminiscent of phage injection machinery. T6SS has diverse roles in virulence, symbiosis, interbacterial interactions, and antipathogenesis in different bacteria (Records 2011). Xcv is found to have two clusters of T6SS; cluster type 1 and cluster type 2, of which the later one is split into two locations. XccA has only cluster type 2 T6SS, which like Xcv is split into two locations (Boyer et al. 2009). The distribution of the T6SS in all three *Xanthomonas* is compiled in Table 2-7. Xacm encodes for two clusters, cluster type one from XACM_2098 – XACM_2121 and cluster type two from XACM_4015 – XACM_3979. Both the clusters are homologues to the ones found in Xcv. XccA on the other hand has only T6SS cluster type two, encoded from XAC4147 to XAC4112.

Bacterial Surface Structures

Lipopolysaccharides (LPS)

LPS is composed of three distinct components; membrane-anchored lipid A, core oligosaccharide and an O-antigen polysaccharide chain. LPS serves a dual role as a physical barrier by protecting the bacteria from antibacterial substances produced by plants and also as inducer of plant defense related genes (Newman et al. 2000). Flanked by highly conserved housekeeping genes for cystathionine gamma lyase (*met*) at one end and electron transport flavoprotein (*etf*) at the other end, the genome of Xacm encodes a cluster of 22 genes that encode genes involved in LPS biosynthesis. The LPS gene cluster encoded by 24.5 kb region in Xacm is markedly different both in gene number and composition as compared to the 17 gene cluster in XccA and more so from the 16 gene cluster in *Xcv* (Fig. 2-11). The flanking genes of *etfB*, *etfA* and *metB/C* are conserved in all the three genomes. The LPS locus of Xacm has at least 6 homologs to XccA and only one to *Xcv* (Fig. 2-11). The LPS cluster is involved in

synthesis of O-antigen polysaccharide. It is known to be important for biofilm formation on the host and contributes to virulence. Two such loci XAC3586 and *rfbC* have been experimentally shown to contribute to virulence of XccA on grapefruit (*Citrus paradisi* cv. Duncan grapefruit) (Li and Wang 2011). Xacm does not have homologs for either of these genes. This may contribute to the poor survival of Xacm in-planta and hence lower virulence as compared to XccA. Interestingly, the O-antigen ABC transporter encoding *wzt* gene mutant of XccA showed more water soaking on citrus plants as compared to wild type strain (Laia et al. 2009). XACM_3499 in Xacm is the closest homolog to *wzt* with a low protein identity of 34% and 41% to its orthologs in XccA and Xcv. Also, the Xacm gene encodes for a truncated protein with half of it missing from the C-terminus region as compared to its orthologs and thus, it might be non-functional. This may have led to variation in symptoms of Xacm and it shows pronounced water soaking as compared to XccA. It was also suggested that there is no obvious correlation of the content of the LPS gene cluster with host specificity (Lu et al. 2008). Thus, the variation in the LPS gene cluster among Xacm, XccA, and Xcv might contribute to their difference in virulence or symptom development in plant hosts rather than serve as a determinant of their differential host range. In either case, these differences are consistent with the diversifying selection based on the changes in this locus put forward by Patil et al. stating that LPS locus in plant pathogenic bacteria shows intense interstrain variation due to horizontal gene transfer (Patil et al. 2007).

Extracellular Polysaccharides

Extracellular polysaccharides (EPS, called xanthan gum in Xanthomonas) are an important component of a biofilm and contributes to epiphytic fitness of *Xanthomonas* spp. (Rigano et al. 2007). It is postulated to promote colonization of plant tissues by

protecting the pathogens from harsh environmental conditions and to contribute to occlusion of vascular tissues in wilts and blights (Kiraly et al. 1997). Xacm encodes for the complete gum gene cluster from *gumA* to *gumP*, which is syntenic to those found in XccA and Xcv. The identity of gum genes ranges from 88-100% among the three Xanthomonas species. Since EPS encoding genes are so conserved, it is unlikely that EPS plays any role in the difference in virulence and host range of Xacm, XccA, and Xcv.

Flagella

Xanthomonas is known to contain all the genes for flagellum synthesis and motility. Various genes located in 4 clusters, which are characteristically flanked by transposase genes, encode the flagellum. Xacm contains complete flagella structure and motility genes in four similar clusters. The flagellar genes in Xacm are mostly organized in similar order to those in Xcv and XccA (Fig. 2-12). Cluster 1 consists of *motA* (XACM_3590) and *motB* (XACM_3592) and cluster 2 of *motB* (XACM_1939) and *motC* (XACM_1940). These genes encode flagellar motor proteins required for rotation of flagella. Cluster 3 in Xacm consists of 24 genes from XACM_1954 to XACM_1977, involved in synthesis and regulation of flagella that is syntenic with XccA and Xcv. The gene *fliK* in this cluster may be a pseudogene, which is non-functional due to frameshift mutations. Mutations in *fliK* affect flagellar hook length in animal pathogenic bacteria (Williams et al. 1996). However, no detectable difference was observed between the motility of XccA and Xacm. Xacm has a cluster comprised of 24 genes from XACM_1991 to XACM_2014, which is highly conserved as compared to both XccA and Xcv.

Interestingly, the genes that lie between these clusters are different in Xacm as compared to XccA. A notable difference includes the absence of homolog of XAC1927 from Xacm. This gene encoding an Fe-S oxidoreductase and located on a probable pathogenicity island, has been linked to virulence in XccA (Laia et al. 2009). The absence of this gene from Xacm could possibly contribute to lower virulence of Xacm pathogen as compared to XccA on citrus.

Regulation of Pathogenicity Factors (Rpf) Cluster

The *rpf* genes control the synthesis of DSF that plays a major role in quorum sensing thus controlling various virulence factors in XccA (Siciliano et al. 2006). Three core genes *rpfF*, *rpfC* and *rpfG* control synthesis of DSF molecule and signal transduction. *rpfF* is responsible for production of DSF, whereas *rpfC*/*rpfG* are two-component signaling factors. RpfC is a sensor protein and RpfG is a response regulator. RpfG has a HD-GYP domain that regulates the amount of cyclic di-GMP. Furthermore this is involved in regulation of the DSF regulon, thus affecting virulence of the pathogen XccA (Andrade et al. 2006). In addition to all the *rpf* genes found in XccA, the CBS pathogen Xacm encodes for a functional *rpfH*, which lies nestled between *rpfC* and *rpfG*. This gene encodes a protein, which is structurally similar to the sensory domain of RpfC. *rpfH* is also present in Xcv and *X. campestris* pv. *campestris* but absent in XccA. A study by Slater et al (Slater et al. 2000) showed that mutation in *rpfH* gene in XccA did not affect the DSF pathway and thus its role in this operon is unclear. It would be interesting to study whether its presence affects virulence of Xacm on citrus.

Other Strain-Specific Genes that Might Contribute to the Distinct Virulence of XccA and Xacm on Citrus

Overall**,** 807 XccA-specific genes were missing in Xacm and Xcv. Besides the genes discussed above, XccA also contains other genes missing from Xacm that may be responsible for its higher virulence. A plant-like natriuretic peptide (XacPNP), which is expressed specifically during the infection process in XccA, is one such gene. XAC2654 encodes this plant-like hormone that induces changes in host photosynthetic efficiency thereby weakening host defense (Garavaglia et al. 2010b). It has been shown that *XacPNP* mimics host PNP and results in improved host tissue health and consequently better pathogen survival in the lesions (Garavaglia et al. 2010a). Xacm genome was found to have neither a homolog of XAC2654 nor the surrounding region in its genome. Interestingly, XccA also encodes for genes with putative toxin producing function. The genes *syrE1* and *syrE2* are similar to those found in *Pseudomonas syringae* encoding the phytotoxin syringomycin (Etchegaray et al. 2004). These non-ribosomal peptide synthetases, which might produce toxins, are absent from Xacm genome. XccA also encodes for haemolysin type calcium binding proteins XAC2197 and XAC2198 along with potential secretion genes *hlyB* and *hlyD*. These genes are also found in the citrus pathogen *Xylella fastidiosa*, and belong to the RTX toxin family (Simpson et al. 2000). They are known to be pore-forming cytotoxins which act as virulence factors and individual toxins often exhibit host-specificity in eukaryotes (Welch 1991). The region containing the toxin genes is absent from Xacm. Another region of 20 kb from approximately 1.72 to 1.74 Mb is specific to XccA and is not found in Xacm. It contains at least two genes XAC1496 and XAC1507 (*mobL*) that are involved in virulence of the canker pathogen (Yan and Wang 2012). This region has a very low

G+C content of 50% and is surrounded by integrase genes suggesting that XccA might have acquired it through recent HGT events. These might be potential genes contributing to higher aggression shown by XccA on citrus as compared to Xacm.

Conclusion

Xanthomonas is a large genus of bacteria that collectively cause disease on more than 300 plant species. The broad host range of the genus contrasts with stringent host and tissue specificity and differences in symptoms for individual species and pathovars. In the present study we conducted a comprehensive comparative genomic study to provide insights into the reduced pathogenicity and limited host range of an aggressive strain F1 of *Xanthomonas axonopodis* pv. citrumelo, causal agent of citrus bacteria spot compared to *X. citri* subsp. citri strain 306, causal agent of citrus canker. To gain a better understanding of the ecological and evolutionary relationships, we also compared Xacm with the closely related *X. campestris* pv. vesicatoria strain 85-10 which causes bacterial spot on tomato and pepper. 454 GS-FLX pyrosequencing, paired-end Illumina/Solexa sequencing and optical mapping was used to obtain high quality finished genome of Xacm which is 4.9 Mb in size. Phylogenetic relatedness based on a set of nine housekeeping genes from completely sequenced *Xanthomonas* indicated that Xacm is closely related to XccA and Xcv forming a clade distinct from other xanthomonads. Comparison of chromosome organization using MAUVE showed inversions (with translocations) and major deletions in Xacm compared to XccA and Xcv. Both XccA and Xcv harbor plasmids pXAC64 & pXAC34 and pXCV183, pXCV38, pXCV19 and pXCV2 respectively, containing various genes involved in pathogenesis. The lack of plasmids in Xacm may have contributed to the reduced virulence of the CBS strain. A sharp drop in the G+C content of various regions in the genome of Xacm

indicated that these may have acquired due to horizontal gene transfer. One of the genomic island postulated to be acquired by HGT possess genes encoding for parts of Type IV secretion system (T4SS), components of Type IV pilus and pilus tip associated proteins. Fifty percent of open reading frames in the two biggest regions of the chromosome of Xacm identified to be acquired by HGT were determined as orphan genes. These orphan genes having a very limited phylogenetic distribution and no recognizable homologs, may encode functional proteins that might contribute to virulence and/or differential host range of the strain. Comparison of the proteomes of three *Xanthomonas* spp. using bi-directional BLASTp revealed that about three-quarters (3,292 CDSs) of the genome of Xacm forms the core set of genes that include conserved house-keeping and virulence genes essential for plant infection. Homologs present in both XccA and Xacm may include virulence factors necessary for infecting citrus host. Higher number of similar homologs between Xacm and Xcv compared to Xacm and XccA confirmed the genetic closeness of CBS strain to Xcv. Xacm genome contain 406 unique CDSs, many of which are hypothetical genes and may contribute to the differences in symptoms and host range. The organization of Type III secretion system (T3SS) gene cluster of Xacm showed close synteny with the *hrp* cluster of XccA and Xcv, however few major differences were also observed. As compared to Xcv, the T3SS of Xacm lacks several *hrp* associated outer protein and hypothetical genes; the loss of which might contribute to the variations in host range and virulence. Considerable differences were observed in the effector repertoires present in Xacm, XccA or Xcv strains. Seventeen effector genes shared by all three strains were defined as core effectors and none of them is likely to be responsible for differences in host

range or virulence among strains. Two species-specific effectors, *xopC2* and *xopW* are postulated to play key role in differential virulence and host range of Xacm. Effectors such as *pthA*, *xopE3, xopAI*, and *hrpW* were absent from Xacm while present in XccA. These effectors might be responsible for survival and the low virulence of this pathogen on citrus compared to that of XccA. The contribution of various effectors in determing host range and differences in virulence needs further characterization. Xacm also encodes for several genes involved in Type I and Type II secretion systems. Xacm is deficient in pectate lyase function (belongs to T2SS) as was confirmed by the lack of pitting in Hildebrands agar medium. Type IV secretion system of Xacm codes for one Vir-type cluster which shows high similarity to *Stenotrophomonas maltophila* rather than XccA or Xcv. Xacm codes for gene *fhaB* (belongs to Type V secretion system) which is likely to be inactive due to the insertion. The product of *fhaB* is a filamentous hemagglutinin protein and the lack of this functional gene could contribute to the low virulence of Xacm compared to XccA, which encodes for a fully functional *fhaB*. Xacm codes for two Type VI secretion system clusters both of which are homologs of T6SS of Xcv. The production of LPS is controlled by 22 genes in a cluster in Xacm which is markedly different in number and composition from XccA or Xcv. Several genes in the LPS cluster shown to contribute to the virulence of XccA are either absent or truncated in Xacm which might explain the variation in symptoms between two strains. The organization of 4 clusters of various genes involved in flagella biosynthesis was similar for all the three strains although differences were observed between the genes which lie between these different clusters. Xacm also lacks various genes, such as *syrE1*, *syrE2*, and RTX toxin family genes which are present in XccA. The absence of these genes

may be associated with distinct virulence of XccA and Xacm. Overall the comparison of the finished genome sequence of Xacm to those of XccA and Xcv provides insights into the emergence of new virulent strains with different host range and distinct virulences. Such knowledge contributes to our understanding of bacterial evolution and the role of various systems in virulence and host range of pathogens. These strain specific genes need to be functionally characterized to understand their roles in virulence and host specificity.

Table 2-1. Overview of sequence data for *Xanthomonas axonopodis* pv. *citrumelo* str. F1

Sequencing method	454 sequencing	Illumina/Solexa
Total reads	367,109	37,695,118
Total sequence output	103,810,015 bp	2,789,438,732 bp
Average read length	332 bp	74 bp
Genome coverage	20	450
No. of contigs[a]	72	1350

Note: 454 contigs assembled using Newbler 2.0 and Illumina/Solexa contigs assembled using CLCbio Genomics Workbench 4.0

Table 2-2. General features of *X. axonopodis* pv. *citrumelo* str. F1 genome

Chromosome features	XACM
Genome Size (bp)	4,967,469
GC content (%)	64.92
Plasmids	0
Protein coding region (%)	86.53
Predicted CDS	
Protein coding genes	4202
with COGs	3087
with Pfam	3293
with TIGRfam	1314
connected to KEGG pathways	1189
Ribosomal RNA	6
rRNA operons	2
Transfer RNA	54

Table 2-3. Functional classification of annotated sequences in genome of *Xanthomonas axonopodis* pv. *citrumelo* F1

COG Categories	Abbreviation	Gene Count	% of Total (3481)
INFORMATION STORAGE AND PROCESSING			
Transcription	K	233	6.69
Translation, ribosomal structure and biogenesis	J	172	4.94
Replication, recombination and repair	L	154	4.42
CELLULAR PROCESSES			
Cell cycle control, cell division, chromosome partitioning	D	33	0.95
Cell motility	N	129	3.71
Cell wall/membrane/envelope biogenesis	M	234	6.72
Posttranslational modification, protein turnover, chaperones	O	148	4.25
Inorganic ion transport and metabolism	P	186	5.34
Signal transduction mechanisms	T	260	7.47
METABOLISM			
Amino acid transport and metabolism	E	231	6.64
Carbohydrate transport and metabolism	G	214	6.15
Energy production and conversion	C	194	5.57
Lipid transport and metabolism	I	136	3.91
Nucleotide transport and metabolism	F	66	1.9
Coenzyme transport and metabolism	H	143	4.11
Secondary metabolites biosynthesis, transport and catabolism	Q	74	2.13
POORLY CHARACTERISED			
Function unknown	S	314	9.02
General function prediction only	R	373	10.72
Chromatin structure and dynamics	B	1	0.03
Cytoskeleton	Z	1	0.03
Defense mechanisms	V	56	1.61
Intracellular trafficking, secretion, and vesicular transport	U	128	3.68
RNA processing and modification	A	1	0.03
Not in COG	—	1196	27.92

Table 2-4. Coordinates, sizes and G+C contents of the segmented domains of *X. axonopodis* pv. *citrumelo* str. F1 genome determined by GC-Profile. The eight potentially horizontally transferred regions are marked with an asterisk. The criteria used for analysis were: Halting parameter = 50; Filtered gap size = 0 bp; Minimum length = 100 bp.

Start (bp)	Stop (bp)	Length (bp)	GC content (%)
1	1051658	1051658	65.58
1051659	1253711	202053	64.01
1253712*	1265138	11427	57.66
1265139	1827506	562368	65.27
1827507*	1891340	63834	55.09
1891341	2417433	526093	64.72
2417434	2438710	21277	68.41
2438711*	2443584	4874	52.11
2443585	2645628	202044	63.80
2645629	2970112	324484	65.38
2970113*	3004362	34250	54.41
3004363	3037313	32951	63.34
3037314	3621975	584662	65.62
3621976	3664589	42614	62.31
3664590*	3686175	21586	57.14
3686176	4550479	864304	65.04
4550480*	4559509	9030	57.70
4559510	4634913	75404	67.92
4634914	4967469	332556	65.49

Table 2-5. Effector repertoire of *X. axonopodis* pv. *citrumelo* str. F1, *X. citri* subsp. *citri* str. 306 and *X. campestris* pv. *vesicatoria* str. 85-10

Effector class	Xacm	XccA	Xcv	Pfam domains	References
Core effectors present in all three strains					
AvrBs2	XACM_0049	XAC0076	XCV0052	Glycerophosphoryl diester phosphodiesterase	(Kearney and Staskawicz 1990)
XopA (Hpa1/HpaG)	XACM_0406	XAC0416	XCV0440	-	(Noel et al. 2002)
XopE1 (AvrXacE1)	XACM_0271	XAC0286	XCV0294	Putative transglutaminase	(Thieme et al. 2007)
XopF2	XACM_2726	XAC2785 Ψ	XCV2942	-	(Roden et al. 2004)
XopI	XACM_0750	XAC0754	XCV0806	F-box protein	(Thieme et al. 2008)
XopK	XACM_3001	XAC3085	XCV3215	-	(Furutani et al. 2009)
XopL	XACM_3007	XAC3090	XCV3220	LRR protein	(Jiang 2007)
XopN	XACM_2728	XAC2786	XCV2944	ARM/HEAT repeat	(Kim et al. 2009)
XopP	XACM_1178	XAC1208	XCV1236		(Roden et al. 2004)
XopQ	XACM_4215	XAC4333	XCV4438	Inosine uridine nucleoside N-ribohydrolase	(Roden et al. 2004)
XopR	XACM_0263	XAC0277	XCV0285	-	(Furutani et al. 2009)
XopV	XACM_0604	XAC0601	XCV0657	-	(Furutani et al. 2009)
XopX	XACM_0532	XAC0543	XCV0572	-	(Metz et al. 2005)
XopZ	XACM_2036	XAC2009	XCV2059	-	(Furutani et al. 2009)
XopAD	XACM_4086	XAC4213	XCV4315 Ψ XCV4314 Ψ XCV4313 Ψ	SKWP repeat protein	(Guidot et al. 2007, Petnicki-Ocwieja et al. 2002)
XopAE (HpaF/HpaG)	XACM_0381	XAC0393	XCV0409 Ψ XCV0408 Ψ	LRR protein	(White et al. 2009)
XopAK	XACM_3563	XAC3666	XCV3786	-	(Petnicki-Ocwieja et al. 2002)

Table 2-5. Continued.

Effector class	Xacm	XccA	Xcv	Pfam domains	References
Effectors shared by Xacm and Xcv but not present in XccA					
XopC1	XACM_2129Σ XACM_2132Σ XACM_2248Σ	-	XCV2435	Phosphoribosyl transferase domain and haloacid dehalogenase-like hydrolase	(Roden et al. 2004)
XopF1 (Hpa4)	XACM_0384	-	XCV0414	-	(Roden et al. 2004)
XopAJ (AvrRxo1)	XACM_4204	-	XCV4428	Zeta toxin	(Zhao et al. 2004)
Effectors shared by XccA and Xcv but not present in Xacm					
XopE2 (AvrXacE3, AvrXccE1)	-	XACb0011	XCV2280	Putative transglutaminase	(Thieme et al. 2007)
Effectors unique to XccA					
PthA (AvrBs3, TAL)	-	XACa0022 (PthA1) XACa0039 (PthA2) XACb0015 (PthA4) XACb0065 (PthA4)	-	Transcriptional activator, nuclear localization	(Algeria et al. 2005)
XopE3 (AvrXacE2)	-	XAC3224	-	Putative transglutaminase	(Nimchuk et al. 2007)
XopAI	-	XAC3230	-	Putative ADP-ribosyltransferase	(Thieme et al. 2005)
HrpW (PopW)	-	XAC2922	-	Pectate lyase	(Park et al. 2006)

Table 2-5. Continued

Effector class	Xacm	XccA	Xcv	Pfam domains	References
Effectors unique to Xcv					
AvrBs1	-	-	XCVd0104	-	(Thieme et al. 2005)
XopB	-	-	XCV0581	-	(Noel et al. 2001)
XopD	-	-	XCV0437	C48-family SUMO cysteine protease (Ulp1 protease family), EAR motif	(Roden et al. 2004)
XopG	-	-	XCV1298	M27 family peptidase clostridium toxin	(Thieme et al. 2005)
AvrBs1.1 (XopH)	-	-	XCVd0105	Putative tyrosine phosphatase	(Thieme et al. 2005)
XopJ1	-	-	XCV2156	C55-family cysteine protease or Ser/Thr acetyltransferase	(Roden et al. 2004)
XopJ3 (AvrRxv)	-	-	XCV0471	C55-family cysteine protease or Ser/Thr acetyltransferase	(Thieme et al. 2005)
XopO	-	-	XCV1055	-	(Thieme et al. 2005)
XopAA	-	-	XCV3785	Early chlorosis factor, proteasome/ cyclosome repeat	(Thieme et al. 2005)
Effectors unique to Xacm					
XopC2	XACM_1180	XAC1209Ψ XAC1210Ψ	XCV1238Ψ XCV1237Ψ	Haloacid dehalogenase-like hydrolase	(White et al. 2009)
XopW	XACM_0435	-	-	-	(Furutani et al. 2009)

Ψ Inactive/Pseudogene

Σ Partial Sequences due to interruption by IS elements during HGT

Table 2-6. Putative Type 2 Secretion System Substrates in *X. axonopodis* pv. *citrumelo* str. F1, *X. citri* subsp. *citri* str. 306 and *X. campestris* pv. *vesicatoria* str. 85-10

Enzymes	Gene	Xacm	XccA	Xcv
Cellulases	*egl1*	XACM_0030	XAC0028	XCV0029
	egl2	XACM_0031	XAC0029	XCV0031
	egl3	XACM_0032	XAC0030	XCV0033
		XACM_0334	XAC0346	XCV0358
	engXCA	XACM_0615	XAC0612	XCV0670
		XACM_1793	XAC3948	XCV1802
	egl4	XACM_2502	XAC2522	XCV2704
	celS	XACM_3403	XAC3507	XCV3634
	bcsZ	XACM_3410	XAC3516	XCV3641
Polygalacturases	*pgl*	XACM_0665	XAC0661	XCV0722
	pglA	--	XAC2374	XCV2571
Rhamnogalacturonase	*rhgB*	XACM_3402	XAC3505	XCV3632
Beta-glucosidase	*bglS*	XACM_1437	XAC1448	XCV1505
	celD	XACM_1816	XAC1793	XCV1823
		XACM_2997	XAC3076	XCV3211
	bglX	XACM_3763	XAC3869	XCV3988
		XACM_4105	XAC4231	XCV4337
Pectate lyase		--	--	XCV2278
	pel	--	XAC2373	XCV2569
	pel1	XACM_2919 pseudogene	XAC2986	XCV3132
	pel2	XACM_3456 pseudogene	XAC3562	XCV3687
Xylanase	*xynC*	XACM_0913	XAC0933/34 partial	XCV0965
		XACM_1262	XAC1286	XCV1335
		XACM_3080	--	XCV3292
	aguA	XACM_4003	XAC4227	XCV4333
	xynA	XACM_4122	XAC4249	XCV4355
	xynB2	XACM_4125	XAC4252	XCV4358
	xynB3	XACM_4127	XAC4254	XCV4360

Table 2-6. Continued.

Enzymes	*Gene*	Xacm	XccA	Xcv
Proteases		XACM_0907	XAC0928	XCV0959
		XACM_0908	XAC0929	XCV0960
	clpA	XACM_2027	XAC2001	XCV2049
		XACM_2704	XAC2763	XCV2918
		XACM_2799	XAC2853	XCV3013
		XACM_3437	XAC3545	XCV3669
	htrA	XACM_3850	XAC3980	XCV4074
		XACM_2775	XAC2831	XCV2993
		XACM_0541	XAC0552	XCV0583
	xcp	XACM_0790	XAC0795	XCV0845
Lipase		XACM_0494	XAC0501	XCV0536

Table 2-7. Putative Type 6 Secretion System clusters in *X. axonopodis* pv. *citrumelo* str. F1, *X. citri* subsp. *citri* str. 306 and *X. campestris* pv. *vesicatoria* str. 85-10

COGs	Xcv 85-10		Xacm F1		XccA 306
	Cluster 1	Cluster 2	Cluster 1	Cluster 2	Cluster 2
3516	XCV2120	XCV4243	XACM_2098	XACM_4015	XAC4147
3517	XCV2121	XCV4242	XACM_2099	XACM_4014	XAC4146
3157	XCV2122	XCV4241	XACM_2100	XACM_4013	XAC4145
4455	XCV2123	XCV4240	XACM_2101	XACM_4012	XAC4144
3518	XCV2124	XCV4239	XACM_2102	XACM_4011	XAC4143
3519	XCV2125	XCV4238	XACM_2103	XACM_4010	XAC4142
3520	XCV2126	XCV4237	XACM_2104	XACM_4009	XAC4141
0542	XCV2127	XCV4236	XACM_2105	XACM_4008	XAC4140
3501	XCV2133	XCV4217	XACM_2111	XACM_3993	XAC4124
3456	XCV2135	XCV4214	XACM_2113	XACM_3991	XAC4122
3522	XCV2136	XCV4211	XACM_2114	XACM_3988	XAC4121
3455	XCV2137	XCV4210	XACM_2115	XACM_3987	XAC4120
3523	XCV2138	XCV4209	XACM_2116	XACM_3986	XAC4119
3913	XCV2139	XCV4208	XACM_2117	XACM_3985	XAC4118
0631	XCV2140	XCV4207	XACM_2118	XACM_3984	XAC4117
0515	XCV2141	XCV4206	XACM_2119	XACM_3983	XAC4116
3515	XCV2143	XCV4202	XACM_2121	XACM_3979	XAC4112

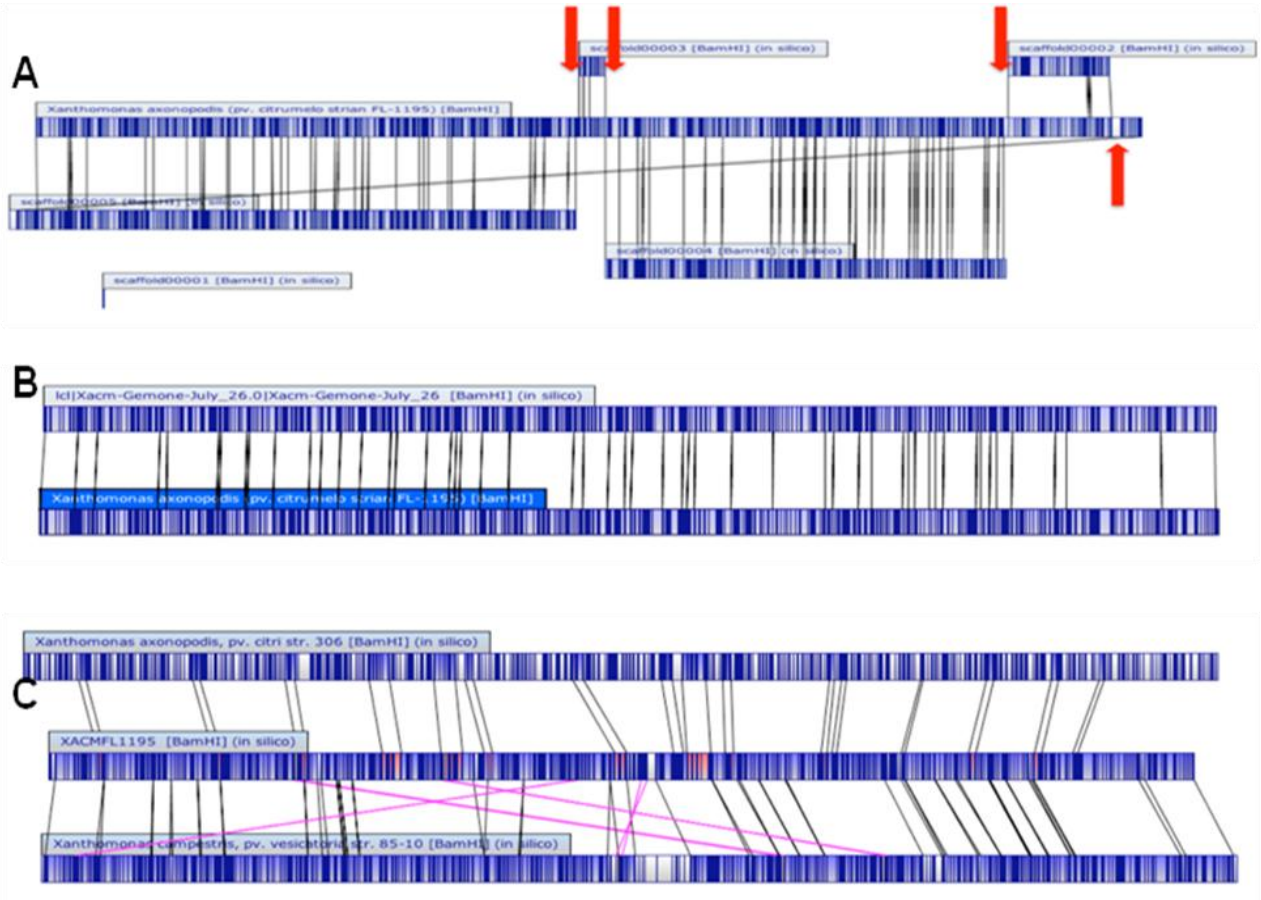


Figure 2-1. Alignments between the whole-genome optical maps and the in silico genome sequence assemblies at various stages of the project. Dark blue represents cut sites, light blue regions indicate alignment, white regions indicate no alignment. A) An early comparison of an optical map derived from *BamH1* digestion of the *X. axonopodis* pv. *citrumelo* F1 (Xacm) genome to the assembled scaffolds generated by traditional sequencing technologies. The Xacm optical map derived from *BamH1* digestion of the chromosome is presented as a single contig in the center. The sequenced genome contains five scaffolds that have a corresponding match to the optical map. Scaffold 1 is too small to be mapped using current optical map technology. However, during gap closure it was placed between contigs 3 and 4. The finishing strategy including gap closure was simplified using the optical map as an assembly model. Red arrows indicate where PCR gap closure was done. B) Comparison of the final assembly of the Xacm genome (top) to the optical map (bottm) for the *BamH1* digest. C) Comparison of the finished sequence of Xacm (center) to the *BamH1* optical map of *Xanthomonas axonopodis* pv. *citri* str. 306 (top) and *Xanthomonas campestris* pv. *vesicatoria* str. 85-10 (bottom). Dark blue represents cut sites, light blue represents aligned regions, red represents regions aligning to both sequences, and white represents unaligned regions. Alignment lines for inversions and translocations highlighted in pink. Inverted and translocated regions highlighted in yellow.

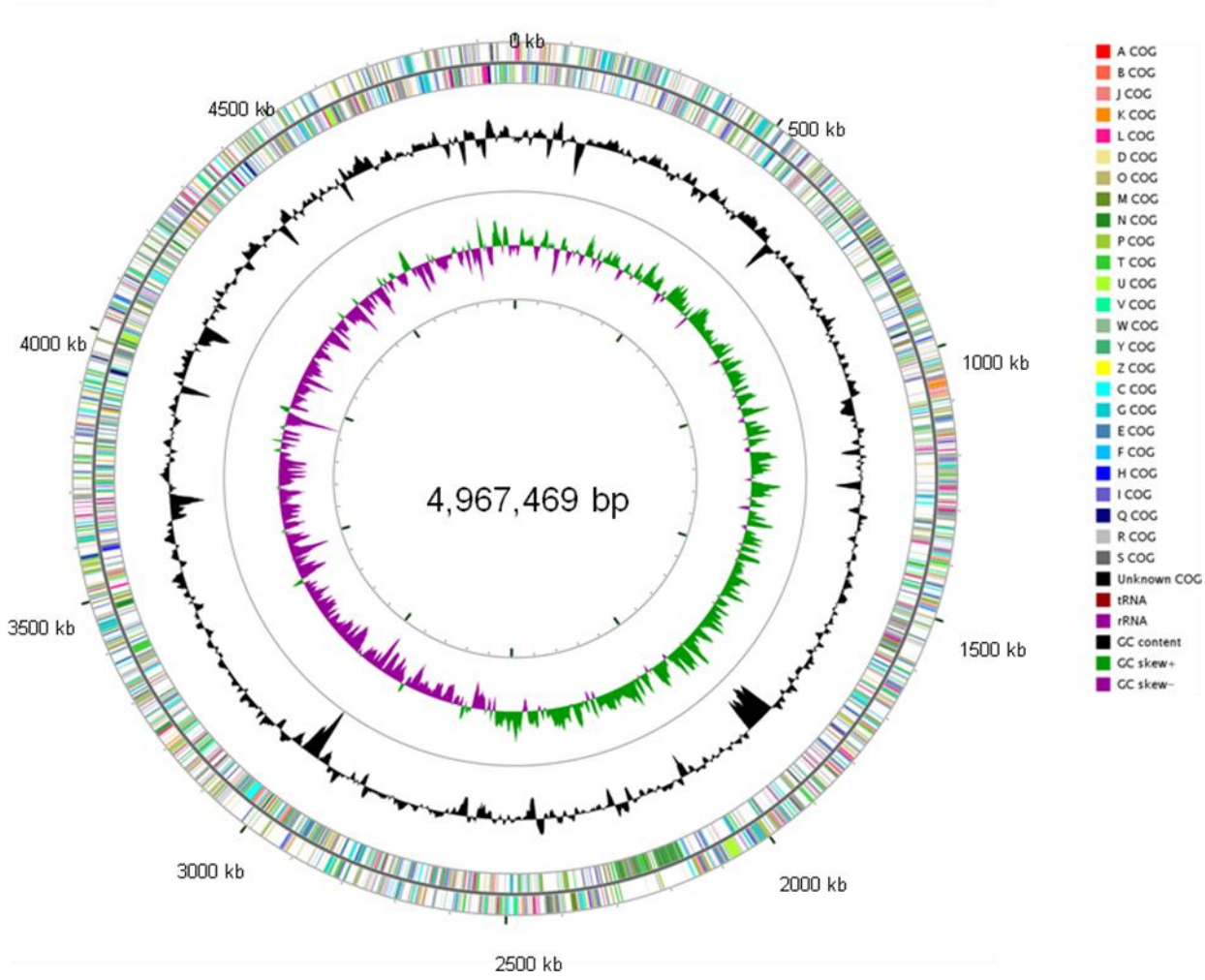


Figure 2-2. Circular representation of *Xanthonomas axonopodis* pv. *citrumelo* F1. Circles from outside to inside: first, scale bar in kilobases; second and third, predicted coding sequences of chromosome on leading and lagging strand respectively (colors according to COGs); fourth, G+C content; fifth, G+C skew.

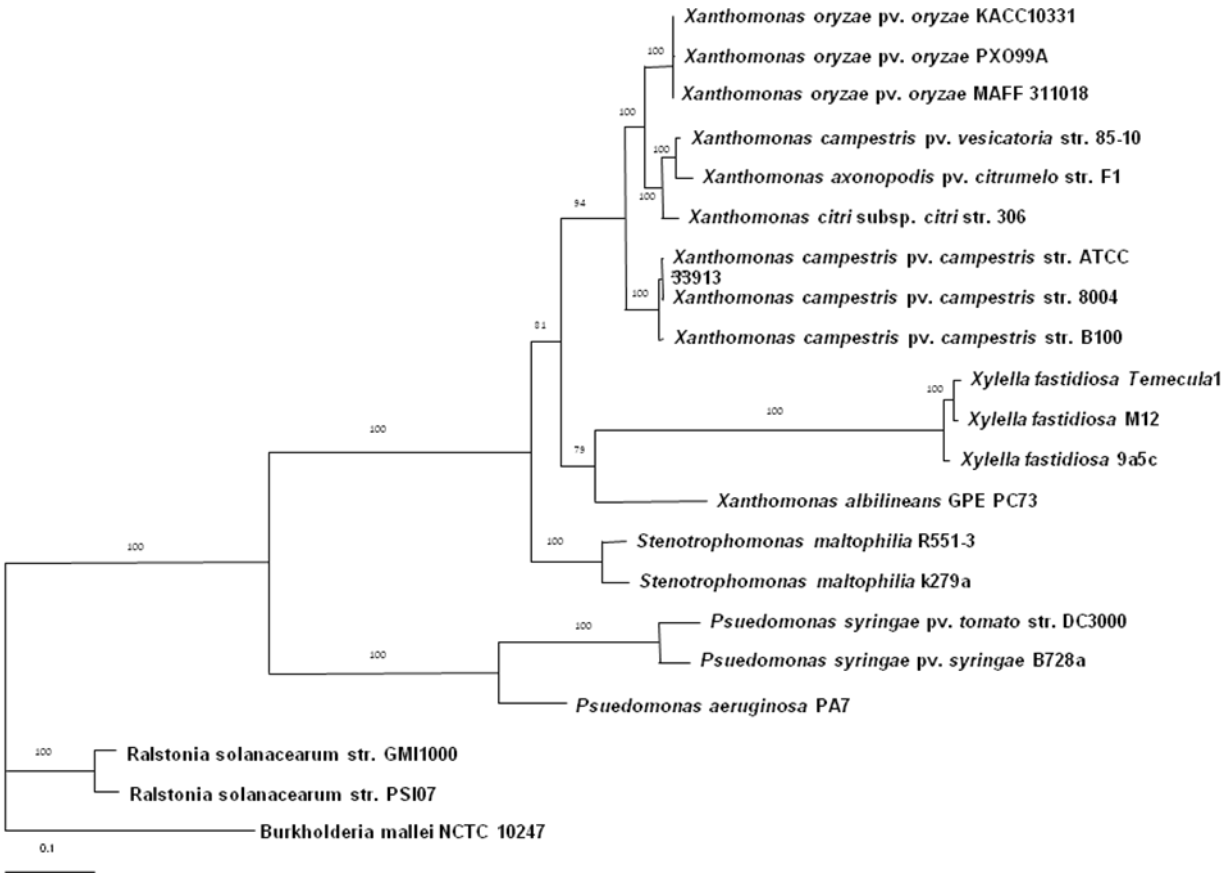


Figure 2-3. Maximum likelihood tree of the genome of *Xanthonomas axonopodis* pv. *citrumelo* F1 showing the relationship to other fully sequenced Xathomonads and related species. The tree was constructed using concatenated protein sequences of nine housekeeping genes (*uvrD, secA, carA, recA, groEL, dnaK, atpD, gyrB* and *infB*) aligned using Clustal W. Phylogenic tree from concatenated sequences was constructed in PAUP (version 4.0) using the Maximum likelihood method. The sequences of *Ralstonia solanacearum* strains GMI1000 & PSI07 and *Burkholderia cenocepacia* strain NCTC 10247 were used as out-group species. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Horizontal scale bar (0.1) at the bottom represents number of amino-acid substitutions per site.

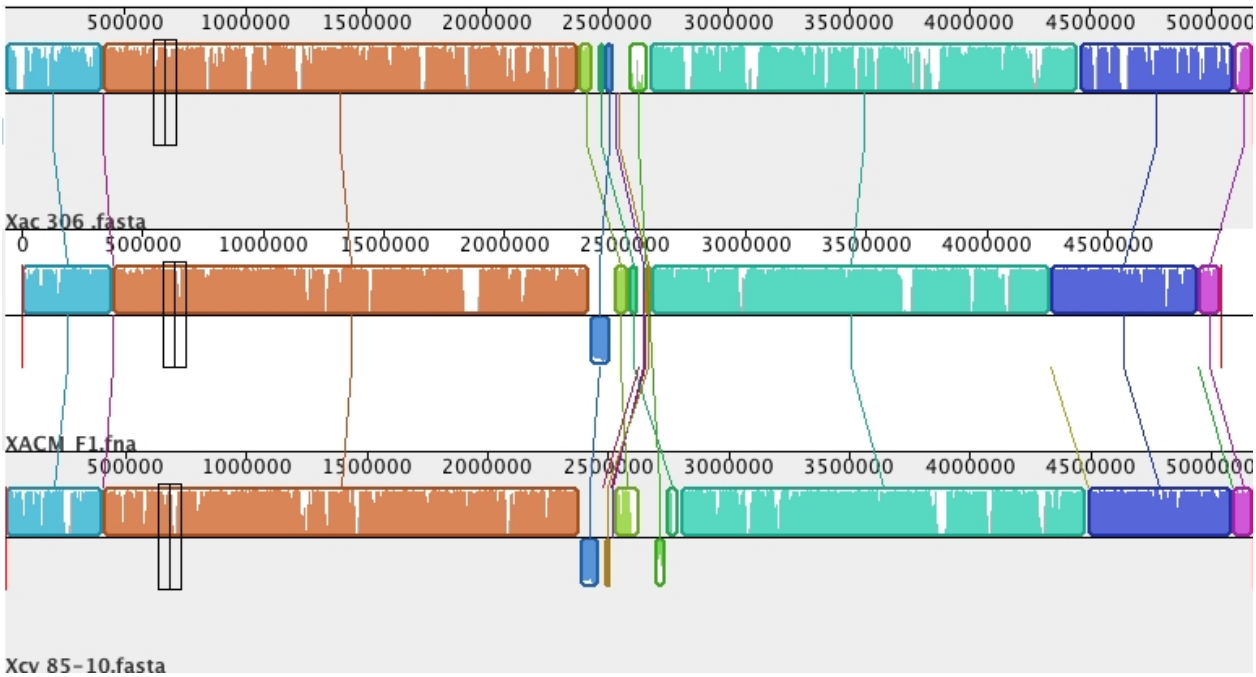
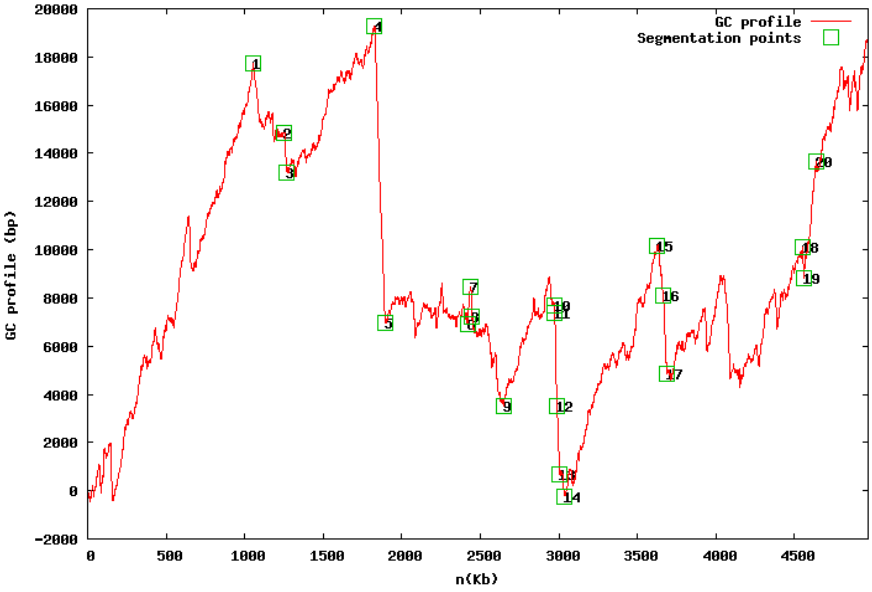
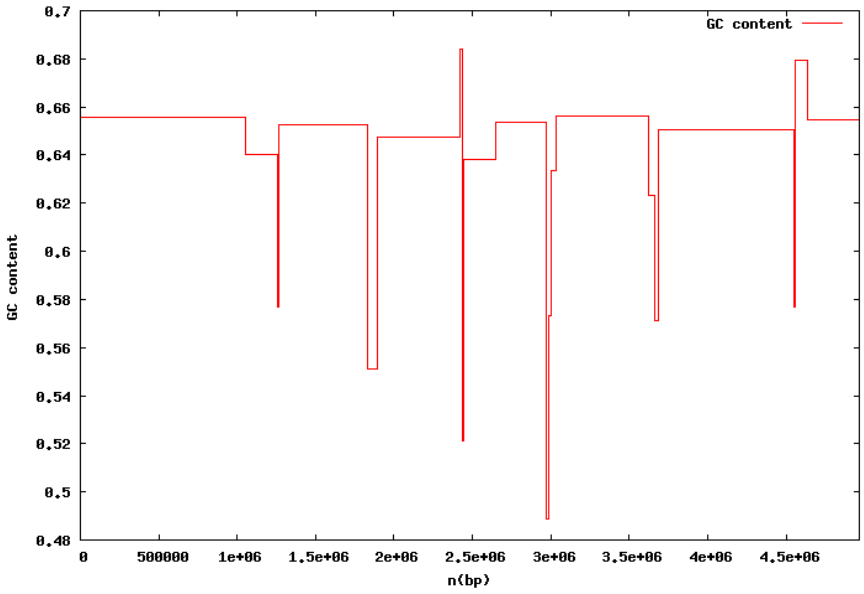


Figure 2-4. MAUVE alignment of the genome sequences of the genome of *X. citri* subsp. *citri* str. 306, *X. axonopodis* pv. *citrumelo* str. F1 and *X. campestris* pv. *vesicatoria* str. 85-10. Conserved and highly related regions are colored and low identity unique region are in white (colorless).



A



B

Figure 2-5. GC profile and GC content of XACM genome. A) The negative cumulative GC profile for the genome of XACM marked with the segmentation points. The segmentation points are obtained at t0= 50. B) Plot representing the distributions of G+C content along the XACM genome. It shows at least six regions of low GC content, which are recognized as genomic island.

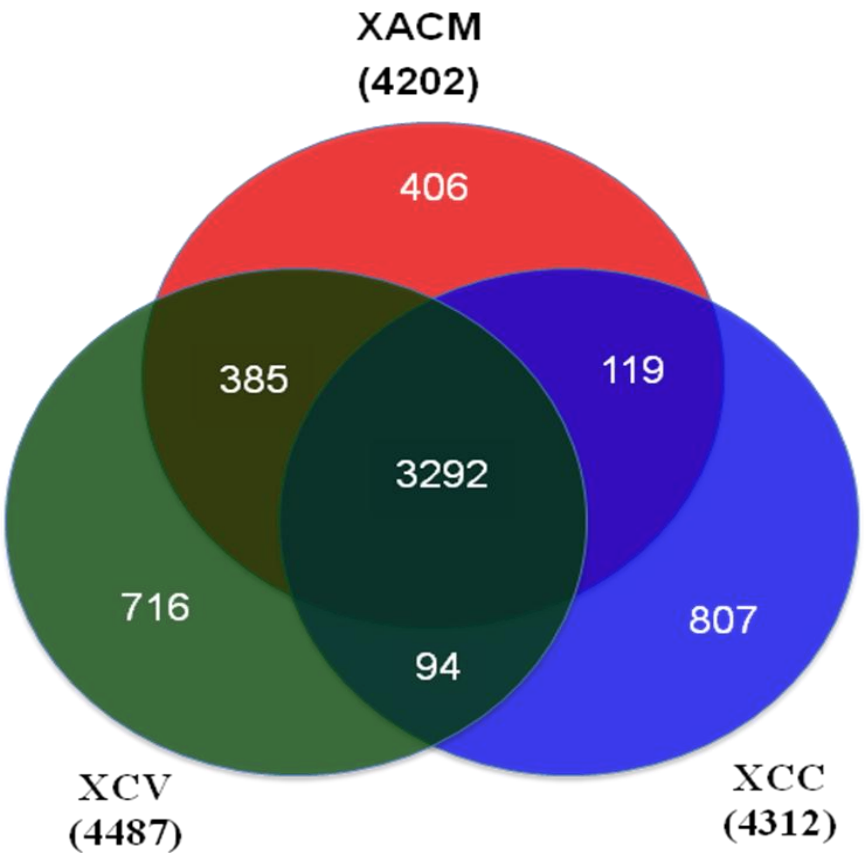


Figure 2-6. Venn diagram representing the pan genome of *X. axonopodis* pv. *citrumelo* F1 (XACM), *X. campestris* pv. *vesicatoria* str. 85-10 (XCV) and *X. citri* subsp. *citri* str. 306 (XCCA). Numbers in brackets represent the protein coding genes on chromosome of each species.

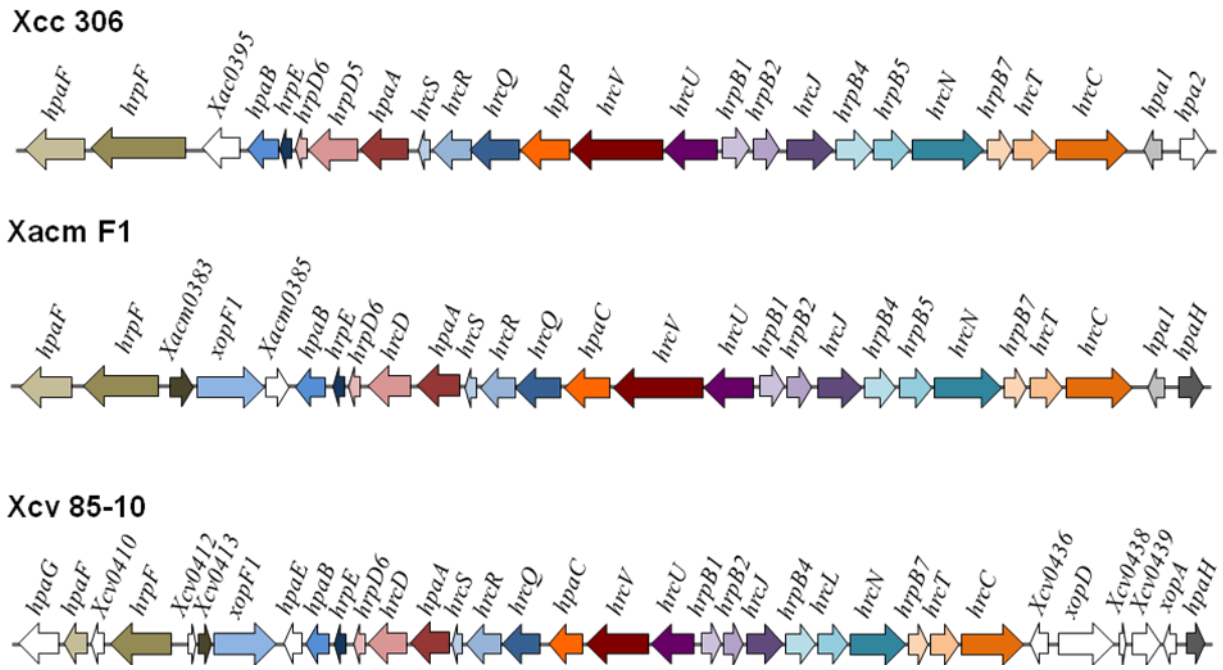


Figure 2-7. Comparison of the hrp gene cluster in the genomes of *X. citri* subsp. *citri* str. 306, *X. axonopodis* pv. *citrumelo* str. F1 and *X. campestris* pv. *vesicatoria* str. 85-10. Arrows indicate individual genes. Homologues genes are colored and low identity or unique genes are white.

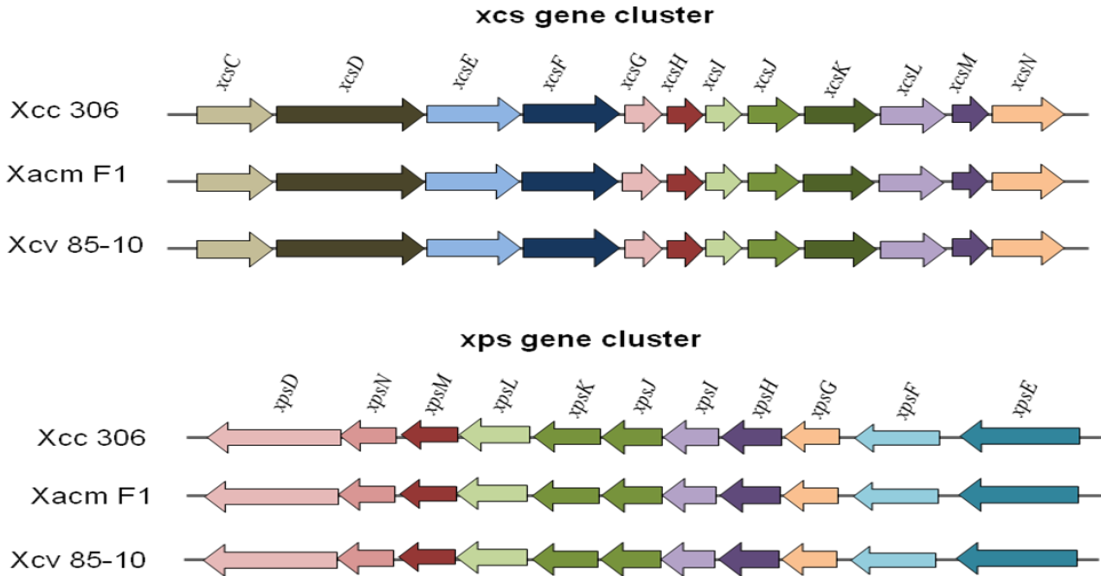


Figure 2-8. Comparison of the xps and xcs gene cluster in the genomes of *X. citri* subsp. *citri* str. 306, *X. axonopodis* pv. *citrumelo* str. F1 and *X. campestris* pv. *vesicatoria* str. 85-10. Arrows indicate individual genes and homologues genes have same color.

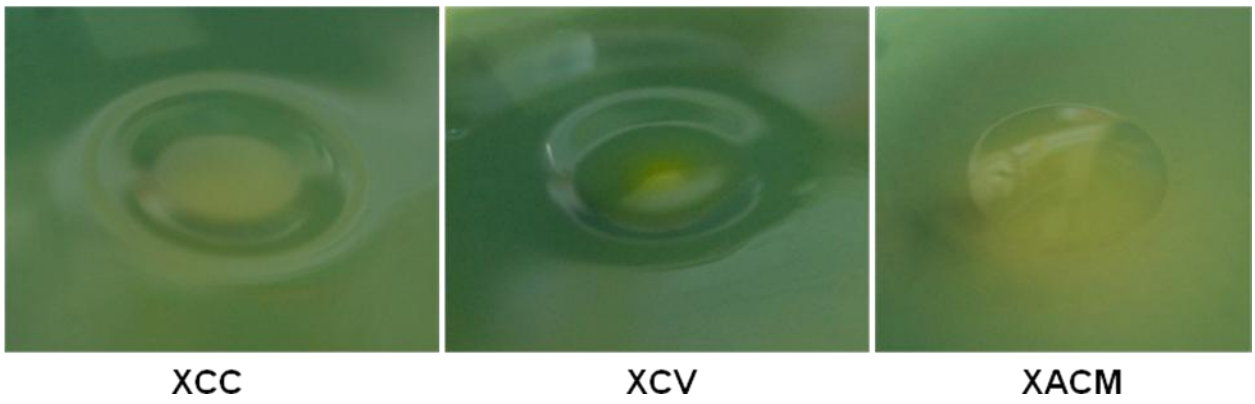


Figure 2-9. Comparison of the pecate lyase production by *X. citri* subsp. *citri* str. 306, *X. axonopodis* pv. *citrumelo* str. F1 and *X. campestris* pv. *vesicatoria* str. 85-10. Pitting can be seen in the Hildebrand's agar medium at pH 8.5 when pectate lyase positive strains are inoculated: XAC and XCV. No pitting is seen for XACM. All strains were incubated in Hildebrand's agar medium at 28°C for 6 days.

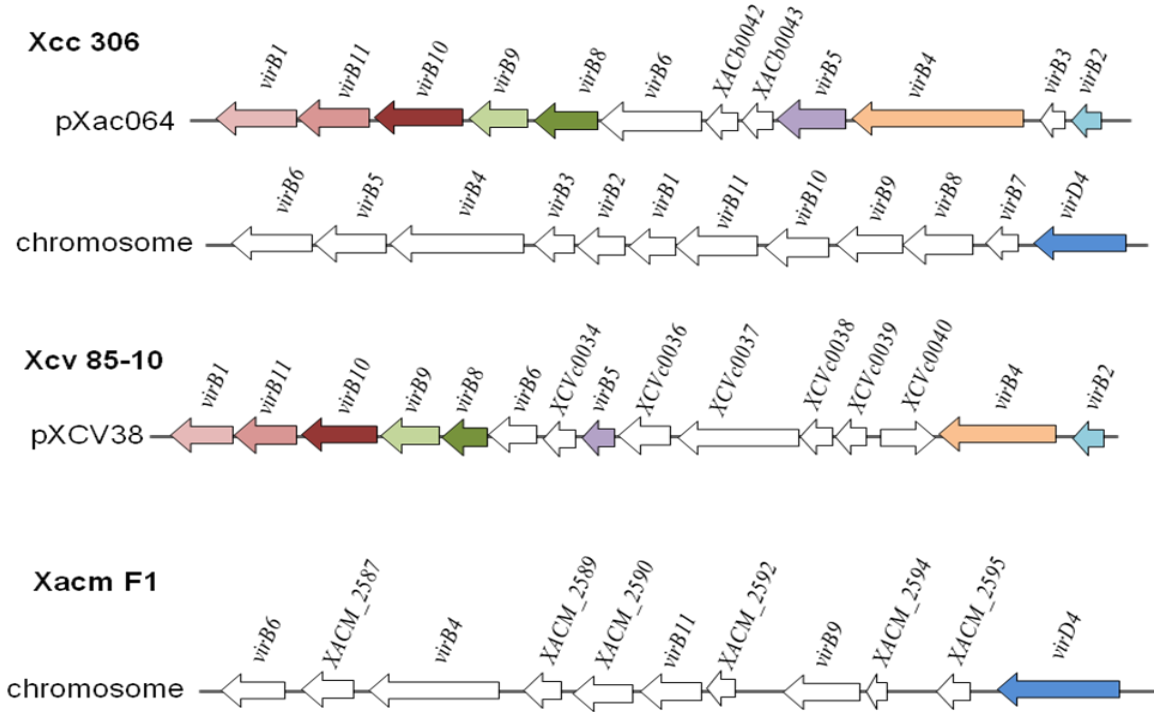


Figure 2-10. Comparison of the T4SS gene clusters in the genomes of *X. citri* subsp. *citri* str. 306, *X. axonopodis* pv. *citrumelo* str. F1 and *X. campestris* pv. *vesicatoria* str. 85-10. Arrows indicate individual genes and homologues genes have same color.

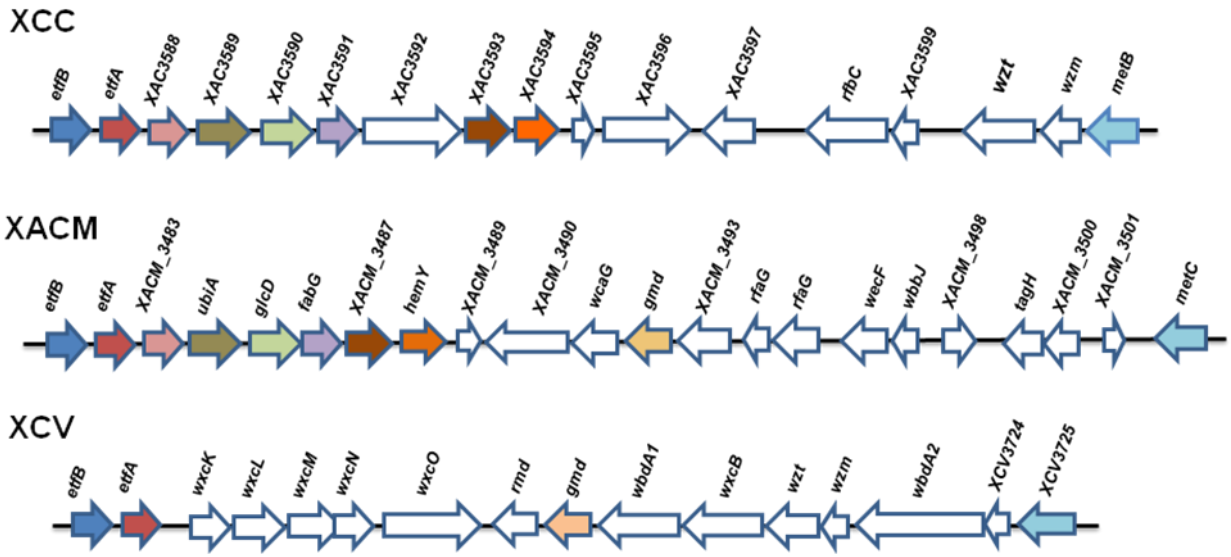


Figure 2-11. Relative organization of the LPS gene cluster in the genomes of *X. axonopodis* pv. *citrumelo* str. F1, *X. citri* subsp. *citri* str. 306 and *X. campestris* pv. *vesicatoria* str. 85-10. Comparison of LPS gene cluster (not to scale). Conserved and highly related genes are colored and low identity or unique genes are white.

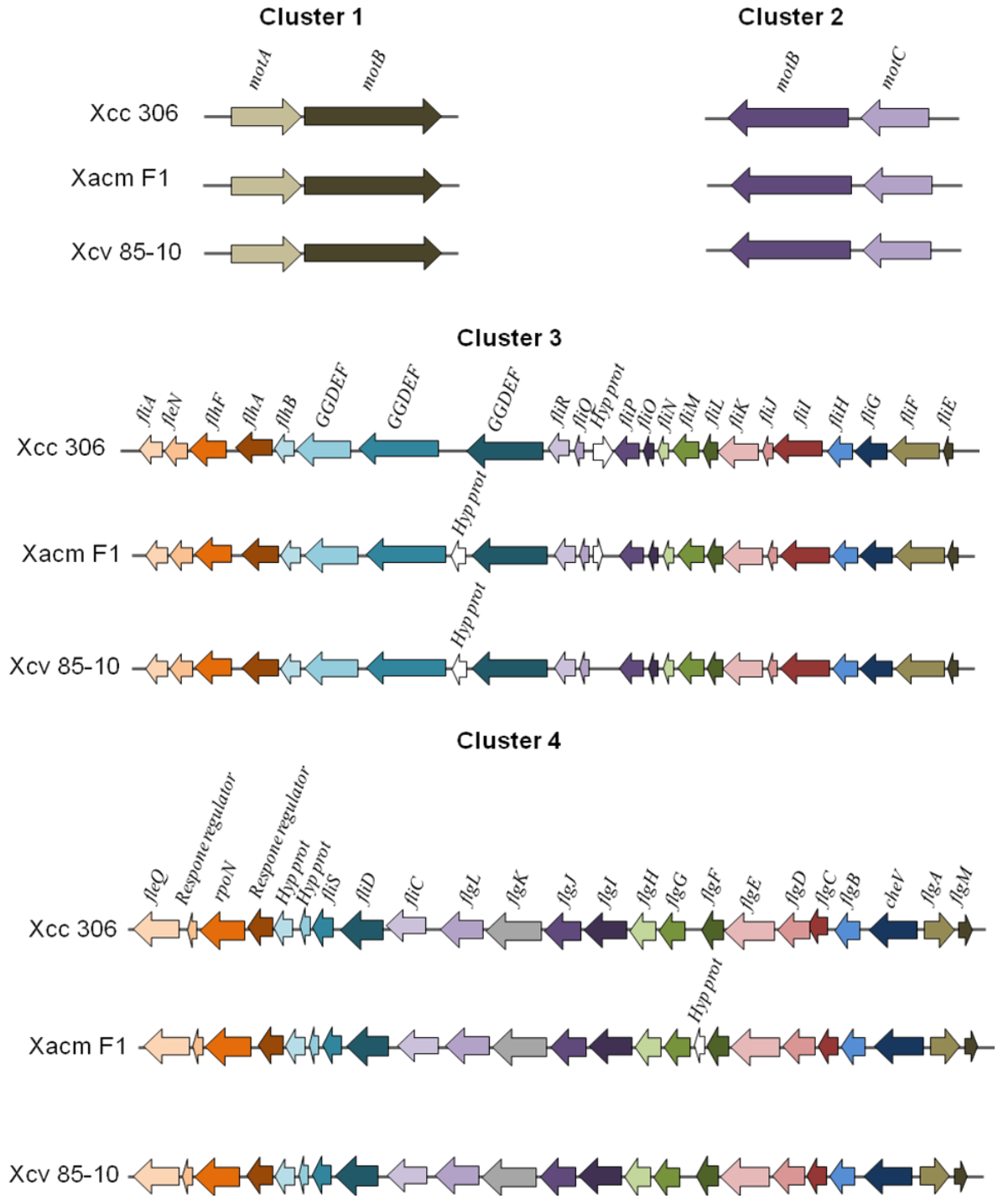


Figure 2-12. Comparison of the flagella gene clusters in the genomes of *X. citri* subsp. *citri* str. 306, *X. axonopodis* pv. *citrumelo* str. F1 and *X. campestris* pv. *vesicatoria* str. 85-10. Arrows indicate individual genes and homologous genes that have same color.

CHAPTER 3
GENE CONTENT OR GENE EXPRESSION, WHICH DETERMINES THE DIFFERENCE IN HOST RANGE AND VIRULENCE OF STRAINS OF *Xanthomonas citri* subsp*. citri*?

Introduction

The co-evolution of plants and microbes is a dynamic process and results from hundreds of millions years of co-existence. Luckily, plants are resistant to most microbes. On the other hand, outbreaks of new diseases have been common and caused disastrous consequences in human history (Plank et al. 1963; McMullen et al. 1997). Those diseases likely resulted from introduction of the pathogens to new areas and to new crops or the endemic pathogens overcoming the plant defense systems. Multiple models have been proposed to describe the co-evolution of plants and microbes (Chisholm et al. 2006; Jones and Dangl 2006; Barrett et al. 2009; Genin 2010). Dramatic efforts have been focused on understanding the mechanisms of how pathogens expand their host range by studying the molecular determinants of virulence and host range of different pathogens (Kay and Bonas 2009; Arnold and Jackson 2011; Ryan et al. 2011; Lindeberg 2012). Knowledge of such mechanisms is critical to prevent, slow down, or prepare for the outbreaks of new diseases.

Xanthomonas is one important model genus for studying the host-microbe interactions and members of this genus are capable of infecting at least 124 monocotyledonous and 268 dicotyledonous plants (Chan and Goodwin 1999). Among the diseases caused by *Xanthomonas*, citrus canker caused by *X. citri* subsp*. citri* (Xcc) is an important disease that has severe economic impact on citrus industries worldwide. Asiatic (A) type canker is the most widespread and destructive form of citrus canker. It produces hyperplasic and hypertrophic (raised) lesions surrounded by oily or water-

soaked margins and a yellow halo on leaves, stems, and fruits. Besides Xcc, *X. fuscans* subsp. *aurantifolii* (Xau) is also known to cause citrus canker with limited geographic distribution and limited host range. XauB is restricted to South America (Argentina, Uruguay and Paraguay) and causes canker B. It mostly affects lemon (*C. limon*) and Mexican lime (*C. aurantifolia*) but is also found on sweet orange (*C. sinensis*) and grapefruit (Civerolo 1984) . XauC is restricted to Brazil and causes canker C only on Mexican lime (Stall 1991).

Compared to Xau, XccA has a broad host range and affects most commercial citrus varieties within the Rutaceae family including grapefruit (*C. paradisi*) and Mexican lime (Verniere 1998; Sun 2004). Two variants of XccA have also been identified. The variant designated A* was found in southeast Asia in the 1990s infecting *C. aurantifolia* (Vernière et al. 1998). The second variant was described by Schubert et al. discovered in Florida in late 1990s (Schubert et al. 2001). This variant designated as the "Wellington strain" was isolated from Palm Beach County in southern Florida and characterized by Sun et al. (2004). This strain of *X. citri* subsp. *citri* strain A^w (Xcaw) was found to be pathogenic to Mexican lime and alemow (*C. macrophyla*) plants, but not to grapefruit and orange. DNA reassociation analysis showed that Xcaw is closely related to XccA and XccA* strains as compared to XauB and XauC strains (Sun et al. 2004). Both Xcaw and XccA cause similar symptoms on Mexican lime and the populations were similar in this susceptible host (Rybak et al. 2009). The Xcaw strain also causes a hypersensitive reaction (HR) in grapefruit (Rybak et al. 2009). AvrGf1 has been shown to be involved in affecting the host range of Xcaw. AvrGf1, an avirulence factor, interacts with the grapefruit host plant and induces the hypersensitive response (HR)

reaction (Rybak et al. 2009). Mutation of *avrGf1* expands the host range of Xcaw to include *C. paradasi*, although the symptoms are much reduced as compared to XccA (Rybak et al. 2009). Thus, it was suggested that Xcaw contains other unidentified factors that are involved in host range limitation. A comprehensive understanding of the molecular mechanisms responsible for the differences in virulence and host range of Xcaw and XccA is lacking.

Comparative genomic analyses of xanthomonads have greatly facilitated our understanding of the suite virulence factors and host range determinants of different pathogens (da Silva et al. 2002; Jalan et al. 2011; Moreira et al. 2011). Comparative genomic analysis of *X. campestris* pv. *campestris* and XccA has been conducted previously to provide a framework for understanding the mechanisms of differing host range and pathogenic processes of the two *Xanthomonas* species which have distinct host specialty (da Silva et al. 2002). Compared to Xcc, which infects citrus and causes citrus canker, *X. campestris* pv*. campestris* affects crucifers such as Brassica and causes black rot. Numerous species-specific genes have been suggested to contribute to the differences in virulence and host range of the two pathogens. Comparative genomic analysis of XccA and *X. axonopodis* pv*. citrumelo* also contributed to the understanding of the mechanisms of bacterial virulence and host specificities. *X. axonopodis* pv*. citrumelo* is the nursery infecting strain and shows low virulence on citrus compared to that of XccA. Differences in gene contents, such as type III effectors (e.g. PthA), the type IV secretion system, and lipopolysaccharide synthesis were identified and may contribute to the differences in bacterial virulence and host range (Jalan et al. 2011; Potnis et al. 2011). Furthermore, sequencing of XauB and XauC

strains revealed the contribution of different virulence factors affecting host range of closely related species (Moreira et al. 2011).

As mentioned above, the previous comparative genomic studies have mainly focused on contribution of the differences in gene content to the differences in virulence and host range (da Silva et al. 2002; Jalan et al. 2011; Moreira et al. 2011; Potnis et al. 2011). The contribution of gene expression due to nucleotide differences in promoter regions and the differences in regulators has been largely ignored. It is known that bacteria coordinate different virulence factors using complex regulatory systems to infect plants. We hypothesized that not only gene content but also gene expression contribute to the differences in virulence and host range of bacterial pathogens.

In this study, we tested our hypothesis by combining comparative genomic and transcriptome analyses to understand the differences in virulence and host range of Xcaw and XccA strains. We completed genome sequencing of *X. citri* subsp. *citri* strain A^w 12879 and compared it with the closely related strain XccA (da Silva et al. 2002). We further examined the transcriptomes of both XccA and Xcaw by Illumina sequencing of cDNAs via RNA-Seq in nutrient rich condition Nutrient broth (NB) and in XVM2, which is known to mimic the conditions in the intercellular space of plant cells (Wengelnik et al. 1996a). Combining comparative genomic and transcriptome analyses provides novel insights into the mechanisms of virulence and host range of pathogens. Our data suggests that both gene content and gene expression contribute to virulence and host range of bacterial pathogens.

Materials And Methods

Bacterial Strain and DNA Sequencing

Genomic DNA was extracted from bacterial culture grown over night at 28°C in NB, using a Wizard DNA purification kit (Promega, USA.) according to the manufacturer's instructions. Quantity and quality of the DNA was measured spectrophotometrically (Nanodrop ND-1000, NanoDrop Tech. Inc., Wilmington, DE). Two high-throughput sequencing techniques, 454 Pyrosequencing and Illumina Solexa GA sequencing were used for whole genome sequencing. Single and paired-end reads were generated on a 454 GS-FLX Titanium sequencer (454 Life sciences, Branford, CT) in accordance with the manufacturer's protocol at Interdisciplinary Center for Biotechnology Research (ICBR), University of Florida. Paired-end Illumina sequence reads were obtained using Illumina Genome Analyzer IIx (Illumina, Hayward, CA, USA) at Yale center for genomic analysis. A *de novo Bam*HI optical map of the genome of Xcaw was generated by OpGen technologies (Madison, Wisconsin, USA). Plasmids were extracted using the Wizard Plus SV Minipreps DNA purification system (Promega, Madison, Wisconsin, USA) and sequenced using 454 GS-FLX titanium sequencer giving paired-end reads.

Data Assembly and Annotation

The total reads obtained by the two sequencing methods (Table 3-1) were trimmed prior to assembly. For *de novo* assembly, the 454 sequencing reads were assembled into contigs using Newbler 2.0 and further grouped into scaffolds using paired-end reads. Illumina reads were assembled using CLC Genomics Workbench (V5.0, CLC Bio) with length fraction and frequency set at 0.8 and all other parameters set as default values. The Illumina contigs were aligned against the 454 scaffolds using

BLASTn to confirm the orientations and integrity of the assembled sequences and to close gaps and link contigs together within the scaffold. *In silico Bam*HI restriction maps of the scaffolds were constructed and aligned to the optical map according to their restriction fragment pattern, using MapSolver v.3.1 software (OpGen Technologies, Inc.). The orientations of scaffolds were corrected as deemed by the alignment and Illumina contigs were used to close the gaps between the scaffolds. Final assembly was correlated with the optical map for further validation. Annotation was done as described by Jalan et al. (2011). In short Softberry's FgenesB suite was used for CDS finding and the predicted proteins were annotated by similarity searches against the NCBI Non-redundant (nr) protein database (http://ncbi.nlm.nih.gov) and clusters of orthologous groups (COG) database. The results of the automated annotation were examined and curated manually using the JGI GenePRIMP pipeline (Pati et al. 2010).

Phylogenetic and Comparative Analysis

The deduced protein sequences of nine housekeeping genes (*uvrD, secA, carA, recA, groEL, dnaK, atpD, gyrB* and *infB*) from 11 completely sequenced *Xanthomonas* spp. were used to determine the position of Xcaw within the evolutionary tree. Additional sequences from two draft *X. aurantifolii* strains and three *Xylella fastidiosa* strains were also used along with *Burkholderia mallei* NCTC 10247 as out-group species. As stated in a previous work by Jalan et al. (2011), amino acid sequences were aligned using clustal W (Larkin et al. 2007). A phylogenetic tree was constructed from the concatenated sequences using PAUP 4.0 (Swafford 2003) by the maximum likelihood method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches in the tree.

For comparative analyses, the sequences of XccA strain 306 (GenBank accession no. NC_003919, NC_003921.3 and NC_003922.1) were retrieved from GenBank. Complete genome sequences of XccA and Xcaw were aligned and visualized in progressive mode using MAUVE (Darling et al. 2010). A two-way BLAST of the protein sequences was done to identify unique genes in each strain. The genes aligned based on amino acid sequence were considered orthologous if reciprocal BLASTp hits was found between two genes with e-value less than or equal to 10^{-20} and alignments exceeding 70% sequence identity and 70% query gene length. A gene was considered singleton or unique to each strain if it had no hits with an *e*-values less than or equal to 10^{-5}.

Preparation of RNA Samples for Transcriptome Analysis

RNA sample preparation and cDNA library generation was performed according to procedures outlined by Filiatrault et al. (2010) with some modifications. RNA samples were extracted from XccA and Xcaw grown to OD560 of 0.4 in XVM2 medium and NB medium at 28°C on shaker at 200 rpm. The starting OD560 for each culture was 0.03. Three biological replicates of each strain in each medium were used for RNA extraction. When the OD560 reached 0.4 for each condition, RNA was stabilized immediately by mixing the culture with two volumes of RNAprotect bacterial reagent (Qiagen, Valencia, CA). The cells were centrifuged at 5000×g at 4°C and cell pellets were treated with lysozyme and RNA extractions were performed using RiboPure bacteria kit (Ambion, Austin, TX) as per manufacturers' instructions. Contaminated genomic DNA was removed by treatment with TURBO DNA-free kit (Ambion, Austin, TX). Total RNA samples were quantified using spectrophotometry (Nanodrop ND-1000, NanoDrop

Tech. Inc.). RNA quality was assessed using the Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA).

mRNA Enrichment and Library Construction

mRNA was enriched from total RNA by complementary oligonucleotide hybridization using MicrobExpress kit (Ambion) with the Pseudomonas module to remove the 23S and 16S ribosomal RNAs (rRNAs). Removal of rRNAs was assessed using an Agilent Bioanalyzer. Double stranded cDNA synthesis was done using the Illumina mRNA Sequencing sample preparation guide method (Cat. No. RS-930-1001) in accordance with the manufacturer's standard protocol. Enriched mRNA was fragmented via incubation for 5 min at 94°C with the Illumina-supplied fragmentation buffer. The first strand of cDNA was synthesized by reverse transcription using random oligo primers. Second-strand synthesis was conducted by incubation with RNAse H and DNA polymerase I. The resulting dsDNA fragments were further end-repaired, and A-nucleotide overhangs were added. After the ligation of Illumina adaptors, the samples were run on a denaturing gel and the band correlating to 200 (±25) base pairs on the denatured DNA ladder was selected. The selected DNA constructs were amplified by PCR using the primers provided in the Illumina library kit. The amplified constructs were purified and the library was validated using Agilent 2100 bioanalyzer.

Illumina Sequencing and Alignment

Paired-end, 75-cycle sequencing of the libraries was performed using an Illumina GAIIx at Yale center for genomic analysis by loading each sample onto a single lane of a flow cell. The raw sequencing reads were further analyzed using CLC Genomics Workbench (V5.0, CLC Bio). The reads were trimmed using the quality score limit of 0.08 and maximum limit of 2 ambiguous nucleotides. The trimmed reads were mapped

as “reference with annotations” to the sequences of XccA strain 306 (GenBank accession no. NC_003919, NC_003921.3 and NC_003922.1) and Xcaw strain 12879, with the parameters allowing mapping of reads to the genome with up to 2 mismatches. The reads mapped to rRNA and the reads not uniquely mapped were removed from further analysis. Gene expression was estimated by calculating read density as ‘reads per kilobase of exon model per million mapped reads’ (RPKM) as described by Mortazavi et al. (2008).

Differential Gene Expression Analysis

The differential gene expression of the pooled samples from each condition was analyzed using CLC Genomics Workbench (V5.0, CLC Bio). RPKM values were normalized using quantile normalization and further $\log_2$ transformed for statistical analysis. Box plots, hierarchical clustering of samples and principal component analysis were done to examine data quality and comparability. A t-test was performed on $\log_2$-transformed data to identify the genes with significant changes in expression between the two growth conditions and between the two strains. The *p-values* were adjusted for the false discovery rate (FDR) using the Benjamini and Hochberg method (1995). Differentially expressed genes were ranked based on FDR, and genes with FDR < 0.05 and $\log_2$ fold-change > 1 were considered as overexpressed whereas those with $\log_2$ fold-change < -1 were down-regulated.

Quantitative Real-Time One-Step RT-PCR

To verify the RNA-Seq result, qRT-PCR assays were carried out using the same sets of RNA for RNA-Seq analysis. Gene specific primers listed in Table 3-2 were designed to generate sequences of 100-250 bp in length from the XccA genome. qRT-PCR was performed for all 3 biological replicates of XccA and Xcaw grown in NB and

XVM2 on a 7500 fast real-time PCR system (Applied Biosystems) using QuantiTect™ SYBR® Green RT-PCR kit (Qiagen) following the manufacturers' instructions. 16S rRNA was used as an endogenous control. The fold change of gene expression was calculated by using the formula $2^{-\Delta\Delta C}{}_T$ (Livak and Schmittgen 2001). The fold change was further $\log_2$ transformed to compare with the RNA-Seq differential gene expression values.

Pathogenicity Assay

Pathogenicity assays were conducted in a quarantine greenhouse facility at Citrus Research and Education Center, Lake Alfred, FL. XccA, Xcaw, and XcawΔavrGf1 strains were grown with shaking overnight at 28ºC in NB, centrifuged down and suspended in sterile tap water and the concentrations were adjusted to 10^8 cfu/ml. The bacterial solutions were infiltrated into fully expanded, immature leaves of Duncan grapefruit, Valencia sweet orange and Hamlin, with needleless syringes (Guo et al. 2011). The test was repeated three times with similar results. Disease symptoms were photographed 5, 10, and 14 days post inoculation (DPI).

Generation of the *xopAF* Mutant and *xopAF, avrGf1* Double Mutant

To construct the *xopAF* deletion mutant, the 1096-bp fragment containing entire *xopAF* gene was amplified using genomic DNA of Xcaw 12879 as template and primers xopAFF1 and xopAFR. This resulted in F1, containing a *Bam*HI restriction site within the *xopAF* gene. A 422bp fragment containing 347bp of *xopAF* gene and its downstream region was amplified further from F1 using primers xopAFF2-*Bam*HI and xopAFR (Table 3-2), resulting in F2. Both F1 and F2 were digested with *Bam*HI and fragments F3 (414bp) and F4 (500bp) were gel purified. The fragments were ligated and cloned into pGEM-T easy vector, resulting in the construct named pGEM-ΔxopAF that was

confirmed by PCR and sequencing. From pGEM-ΔxopAF, an *Apa*I-*Pst*I fragment containing *xopAF* gene with internal deletion was transferred into *Apa*I-*Pst*I digested suicide vector pNTPS138, resulting in pNTPS-ΔxopAF. The construct pNTPS-ΔxopAF was transformed into *E. coli* DH5αλPIR. The construct was purified from *E. coli* and subsequently transferred into Xcaw12879 and XcawΔavrGf1 generated in a previous study (Rybak et al. 2009) by electroporation. Transformants were selected on NA medium supplemented with Kanamycin. Positive colonies were replicated on both NA plates supplemented with 5% (w/v) sucrose and Kanamycin, and only NA and Kanamycin. The sucrose sensitive colonies were selected from NA plus Kanamycin plate and grown in NB medium overnight at 28ºC. The culture was then dilution-plated on NA containing 5% sucrose to select for resolution of the construct by a second cross-over event. The resulting deletion mutant of *xopAF* and double mutant of *xopAF* and *avrGf1* was confirmed by PCR.

Growth Assay *in planta*

XccA, Xcaw, XcawΔxopAF, XcawΔavrGf1 and XcawΔxopAFΔavrGf1 strains were grown with shaking overnight at 28ºC in NB, centrifuged down and suspended in sterile tap water and the concentrations were adjusted to 10^6 cfu/ml. The bacterial solutions were infiltrated into fully expanded, immature leaves of Duncan grapefruit, Mexican Lime and Valencia sweet orange with needleless syringes (Guo et al. 2011). To evaluate the growth of various Xcc strains and mutants in these plants 2 inoculated leaves were collected from each plant at 0, 2, 4, 7, 10, 14 and 21 days. 1 cm^2 leaf disks from inoculated leaves were cut with a cork borer and then ground in 1 ml sterile water. These were serially diluted and plated on NA plates. The bacterial colonies were

counted after 3-day incubation at 28ºC.The test was repeated three times independently.

Pectate Lyase and Proteinase Assay

Xcc and Xcaw were grown on nutrient agar at 28°C, then suspended in sterile deionized water to the O.D. of 0.3 at 560nm. Hildebrand's medium A, B and C were used to test for pectolytic activity (Hildebrand 1971). In short the medium contained bromothymol blue dye, calcium chloride, 2% sodium polypectate and 0.4% agar. The pH was adjusted to 4.5, 7.0 and 8.5 for the medium A, B and C. One µl of the cultures were inoculated onto the plates and incubated at 28°C for 6 days before confirming pitting due to pectate lyase production. 10% skim milk agar was used to test the bacterial protease activity. The cultures were grown and suspended in sterile water as explained above. One µl of the cultures were inoculated onto the skim-milk plates and cultured at 28°C for 6 days to observe protease activity.

Data Access

The genome sequences of Xcaw are available at GenBank under the accession numbers CP003778, CP003779 and CP003780. The RNA-Seq data from this study are available in the NCBI's Gene Expression Omnibus database under the accession number GSE41519.

Results

Genome Sequencing of Xcaw

Xcaw strain 12879 was isolated from Palm Beach county, Florida prior to 2004 (Sun et al. 2004). To generate a high quality finished genome we combined two independent sequencing approaches; 454 and Illumina sequencing. Table 3-1 shows an overview of the reads from both the technologies that were assembled into contigs

separately. The 378 contigs generated by 454 Pyrosequencing were grouped into 17 scaffolds based on paired-end reads. *De novo* assembly of solexa reads yielded 1,426 contigs, which were used to confirm the 454 Pyrosequencing scaffolds and close the gaps within them. The 17 scaffolds were further aligned to the *Bam*HI optical map that revealed several misassembles and changed orientation of the scaffolds (Fig. 3-1A). The changes were made as per the optical map and gaps between the scaffolds were closed using the 454/Illumina contigs and by primer walking. The complete genome was further validated by manually checking all areas of imperfect match between the optical map and the sequence assembly (Fig. 3-1B).

Table 3-3 shows the genome features of Xcaw12879, which is comprised of a single circular chromosome of 5.3 Mb and two plasmids pXcaw19 and pXcaw58 of approx. 19 kb and 58 kb, respectively (Fig. 3-2). The genome consists of 4,675 annotated protein coding sequences (CDS) and 54 structural RNAs (Table 3-3). After annotation and manual curation, 3,423 CDSs could be assigned to one or more COG functional classes whereas 1,252 could not be assigned to any COG category.

Multilocus sequencing test (MLST) based phylogenetic analysis was performed for Xcaw12879 and other *Xanthomonas* using 9 housekeeping genes (*uvrD, secA, carA, recA, groEL, dnaK, atpD, gyrB*, and *infB*) that are highly conserved in bacteria. We aligned the nine nucleotide sequences, concatenated them and constructed a maximum-likelihood phylogenetic tree (Fig. 3-3). We ascertained that Xcaw is closely related to XccA. The two Xcc strains, which are the most closely related, form a separate clade from the other two citrus canker causing bacteria, XauB and XauC.

Chromosome Organization and Genome Plasticity

The genome of Xcaw shows the presence of insertion sequence (IS) elements, phage related genes and plasmids, which are all important sources for genome evolution of bacteria (Mira et al. 2002). Whole-genome alignment of Xcaw to closely related XccA using MAUVE in progressive mode revealed many inversions and translocations (Fig. 3-4). Most of the separated blocks in the alignment are associated with integrases and/or IS elements on at least one of their borders. The IS elements have been known to aid horizontal gene transfer and other genome rearrangements as seen in the alignment above. The Xcaw genome also shows various insertions and deletions throughout the genome as seen in Fig. 3-4.

Xcaw12879 genome consists of two plasmids pXcaw19 and pXcaw58 that are significantly different from the plasmids found in XccA. Plasmid pXcaw19 sequence has no homology with the plasmids of XccA 306, whereas pXcaw58 is only about 35% similar to pXAC64. Plasmid pXcaw58 consists of *pthAw2* gene, a homolog of *pthA4*, which is capable of conferring the ability to cause canker-like symptoms (Al-Saadi et al. 2007). However, the plasmid pXcaw58 does not contain the Vir like type IV secretion system genes found on pXAC64 (Fig. 3-5). The type IV secretion system has been shown to contribute to virulence in *X. campestris* pv. *campestris* strain 8004 (Qian et al. 2005) and absence of these genes from the plasmid could affect virulence of Xcaw strain.

The BLASTp analysis of all the proteins from Xcaw and XccA revealed various gene clusters specific to each strain. Of the 4,760 proteins from Xcaw and 4,427 proteins from XccA, 4,034 proteins are found to be orthologous using the cut-off, e-value ≤ 10^{-20} and alignments > 70% sequence identity and, > 70% query gene length.

Xcaw has 726 proteins that are either are non-orthologous to proteins from XccA, whereas XccA has 393 such proteins.

The *hrp* and *hrc* genes encoding the TTSS in Xcaw are homologous to the *hrp* and *hrc* genes found in XccA. All the genes are found in similar order with the exception in gene annotation between *hrpF* and *hpaB*. The genome of XccA contains the annotated gene, XAC0395, between the two, which is a hypothetical protein. The annotation in Xcaw in the same region is on the opposite strand and contains *hpaI* (XCAW_00803) and pseudogene *xopF1* (XCAW_00804/XCAW_00805). The nucleotide sequences in both strains are same and the differences in annotation were confirmed by BLAST similarity of the annotated genes in Xcaw to other xanthomonads. Our RNA-Seq data supports our annotation of *hpaI* and *xopF1* (data not shown).

The TTSS translocates effector proteins into the plant cells. These proteins further cause disease in plants via different mechanisms (Büttner and Bonas 2010). The effectors can either aid in nutrient acquisition and virulence or act as avirulence factors that trigger host immune response. The type III effector genes in Xcaw were predicted by BLAST analysis against the known TTSS effector database (http://www.xanthomonas.org). The TTSS effectors of Xcaw showed notable differences in comparison to effectors in the other three citrus canker causing strains XccA, XauB, and XauC as summarized in Table 3-4.

Xcaw contains thirty-two effector genes of which nineteen are present in all four sequenced citrus canker causing variants (XccA, Xcaw, XauB, and XauC) compared and thus represent the core effector set for *Xanthomonas* that cause citrus canker. The effector genes *avrBs2, xopK, xopL, xopQ, xopR, xopX* and *xopZ* are found in all other

sequenced *Xanthomonas* genomes and hence the 7 genes might be a core set of effectors required for phytopathogenicity as suggested by Moreira et al. (2010). Twelve effector genes (*xopA, xopE1, xopE3, pthA4* or its functional homologs*, xopI, xopV, xopAD, xopAI, xopAK, xopAP, hpaA*, and *hrpW*) are present in all four genomes that were compared (Xcaw, XccA, XauB and XauC). Thus these effectors might be necessary for causing disease on citrus host but not on other hosts. Some of these 12 effectors might act as avirulence genes in other hosts and activate the plant immune system. Two effector genes *avrGf1* and *xopAF* were identified in Xcaw, XauB and XauC but were not present in XccA genome (Table 3-4).

Multiple genes clustered into 9 groups were identified in Xcaw but not in XccA (Table 3-5). Most genes of the clusters except cluster 1 of Xcaw have homologs with other *Xanthomonas* species. These regions contain transposase, integrase or phage related genes indicating horizontal gene transfer. The most prominent difference noted in the above-mentioned regions is cluster 8, which encodes for lipopolysaccharide (LPS) biosynthetic pathway. Interestingly, the LPS cluster in Xcaw is chimeric which contains regions orthologous to both XccA and *X. oryzae* pv. *oryzicola* BLS256 as shown in Fig. 3-9, which indicates that there has been horizontal gene transfer. The Xcaw unique clusters also encode large numbers of phage related genes (Table 3-5).

Pathogenicity and Growth Assays

The three strains of *Xanthomonas* affecting citrus, Xcaw, XauB and XauC but not XccA have either complete *avrGf1* or partial sequence and *xopAF* genes in their genomes. The gene *avrGf1* has been earlier studied in Xcaw and is known to be responsible for HR in grapefruit (Rybak et al. 2009). However its effect on other varieties of citrus such as sweet orange is unknown. Also, since *xopAF* is the other

putative effector gene its effect on host limitation was further characterized by pathogenicity and growth assays of XcawΔxopAF and XcawΔavrGf1ΔxopF1.

Pathogenicity assays indicated that Xcaw did not elicit a reaction on Valencia or Hamlin while wild type strain XccA caused typical necrotic raised lesions typical of citrus canker on the leaves at a high bacterial inoculation concentration of 10^8 cfu/ml (Fig. 3-7). Xcaw showed a hypersensitive reaction on Grapefruit leaves that was abolished by deleting *avrGf1* gene (XcawΔavrGf1), however the growth of the mutant was visibly reduced as compared to XccA strain. XcawΔavrGf1 did not show any symptoms or reaction on either Valencia or Hamlin (Fig. 3-7)

To check whether mutation of *xopAF* affects Xcaw growth *in planta*, the wild-type strain XccA, Xcaw, XcawΔxopAF, XcawΔavrGf1 and XcawΔxopAFΔavrGf1 mutants were inoculated into Grapefruit, Mexican Lime and Valencia leaves. As shown in Fig. 3-8A, the population of Xcaw is much lower as compared to XccA in grapefruit. This is restored to some extent in XcawΔavrGf1, which causes symptoms on grapefruit. However, the populations of XcawΔxopAF and XcawΔxopAFΔavrGf1 mutants were one order magnitude lower than that of Xcaw and XcawΔavrGf1 respectively, indicating that mutation of *xopAF* gene has slowed the growth of Xcaw *in planta*. Similar trend was observed in Mexican Lime where the growths of *xopAF* single and *xopAF avrGf1* double mutants were lower as compared to Xcaw and XcawΔavrGf1 respectively (Fig. 3-8B). No significant changes were observed in Valencia leaves as neither Xcaw nor any of its mutants grew well in the sweet orange variety as compared to XccA (Fig. 3-8C).

Transcriptome Analysis of Xcaw and XccA Under Nutrient Rich (NB) and Plant Intercellular Space Mimicing (XVM2) Conditions

To determine the differential gene expression amongst the strains of *X. citri* subsp. *citri*, we grew Xcaw and XccA under nutrient rich condition in Nutrient Broth (NB) and in XVM2 that mimics the plant intercellular growth environment (Wengelnik et al. 1996a). Three biological replicates of the strains were used to collect cells at O.D. 0.4 (Fig. 3-10) and extract the total RNA, enrich for mRNA and sequence cDNA for RNA-Seq. Over 45 million reads were obtained on average for each sample. After trimming and mapping approximately 96% of the reads were mapped to the genomes (data not shown) indicating that RNA-Seq provides high quality reads suitable for *Xanthomonas* transcriptomics. Of all the reads over 6.5 million could be mapped from each sample to mRNA specifically (Table 3-6) giving the enrichment of mRNA up to 28.5% or less. Overall, the three biological replicates gave an average coverage of approximately 98 times for each gene, thus resulting in deep sequencing of the Xcc transcriptome which could cover majority of the genes transcribed. To quantify the expression of each gene, the reads aligned to each gene were pooled and normalized for gene size by calculating the RPKM values (reads per kilobase CDS length per million reads). The values for each gene from all the replicates were further quantile normalized to test them statistically. The resulting values were $\log_2$ transformed and t-test was performed on these expression values to compare differential gene expression (DGE) between XccA and Xcaw under the same growth conditions or between the same strains in NB or XVM2 growth conditions. High correlation was observed between differential expression values of biological replicates (Table 3-7) signifying that the method was reproducible.

Principal component analysis indicates that the biological replicates of XccA formed a separate cluster from Xcaw in both the growth conditions (Fig. 3-11).

One-step quantitative RT-PCR (qRT-PCR) was used to validate the RNA-Seq data. Eight genes were chosen (Table 3-2) which were differentially expressed in Xcaw as compared to XccA under both NB and XVM2 growth conditions to compare data obtained from the two methods. The resulting transcriptional ratio from qRT-PCR analysis was $\log_2$ transformed and t-test was performed to compared with the DGE values obtained by RNA-Seq (Fig. 3-12). Although the scale of fold changes between the two techniques is different, high correlation coefficient of 0.87 verifies that the general trend of gene expression is consistent for both the data sets.

We studied the expression profile of Xcc strains in XVM2 as compared to NB. At the cut-off of $\log_2$ fold change = 1.585 ($|\text{fold change}| = 3$), FDR<0.05, 292 genes showed differential expression (173 up-regulated and 119 down-regulated in XVM2 compared to NB) in XccA (Table 3-8) and 281 genes (129 up-regulated and 152 down-regulated in XVM2 compared to NB) for Xcaw (Table 3-9). The entire TTSS cluster consisting of 25 genes except one gene (XAC0395) was up-regulated in XVM2 for both XccA and Xcaw strains ascertaining that XVM2 is an excellent *hrp* inducing medium (Tables 3-8 and 3-9). Among all the effectors, 16 were induced for XccA whereas 19 effectors were overexpressed for Xcaw in XVM2. As identified in this study, the effectors *avrBs2, xopA, xopE1, xopE3, xopI, xopX, xopZ1, xopAD, xopAP, xopAQ, hpaA, xopN* and *xopP* were up-regulated in both strains while *pthA1*, *pthA2*, *avrXacE3* and *xopK* were induced only in XccA and *xopL, xopR, xopAI, xopAK, xopAF* and *xopAG* only in Xcaw strain. In addition, one putative effector gene was identified in XccA in this study.

XccA contains one currently unannotated gene encoding XopAQ between XAC3223 and XAC3224. This region was identified as differentially expressed intergenic region in XVM2 medium as compared to NB. An open reading frame was identified at this region which showed $\log_2$-fold change of 1.81 ($|\text{fold change}| = 3.51$) (Fig. 3-13). BLAST analysis revealed that the ORF encodes for putative XopAQ effector protein, 100% identical to XCAW_03514 in Wellington strain and 85% identical to XGA_2091 from *X. gardneri* (Potnis et al. 2011). Further BLAST analysis also revealed presence of this ORF in XauB located between XAUB_14670 and XAUB_14680.

The 11-gene *xps* cluster encodes for type II secretion system (T2SS) in *Xanthomonas* secreting various enzymes including pectate lyase, cellulase, and xylanase. The *xps* genes were down-regulated in XVM2 as compared to NB for Xcaw with *xpsE* being the most significantly down-regulated with $\log_2$ fold change -1.07 at FDR = 0.03. XpsE is known to be a key component of T2SS, the loss of which leads to lower virulence in *X. oryzae* (Sun et al. 2004). For XccA, the *xps* genes were not down-regulated. Besides the TIISS genes, at least 22 genes encoding TIISS substrates in XccA were overexpressed in XVM2 as compared to only 12 in Xcaw. To the contrary 11 genes for Xcaw and 8 for XccA were down-regulated (Tables 3-8; 3-9).

Our analysis showed that all the flagella biosynthesis genes encoded by *flg* and *fli*, motility by *mot* and chemotaxis by *mcp*, *che* and *tsr* were repressed in XVM2 for XccA and Xcaw except *cheY* (XAC3284 in XccA and XCAW_03412 in Xcaw) and *tar* (XCAW_03417, XCAW_04009 and XCAW_02497). The genes encoding LPS were down-regulated in both strains, whereas the xanthan gum (EPS) genes were overexpressed in both except *gumP* in XccA. This is in concurrence with the infection

cycle where the bacterial motility will be suppressed to help it attach and colonize with the help of extracellular polysaccharides. LPS also acts as a pathogen-associated molecular pattern (PAMP), and might be down-regulated *in planta* to avoid bacterial recognition. A few genes encoding outer membrane proteins, which help in adhesion, including *ompW*, *blc* and *hms* were up-regulated in XVM2 as compared to in NB for both strains while *xadA* and *yapH* were induced in XccA but down-regulated in Xcaw. The Type IV pilli genes encoded by *pil* and *fim* genes except *pilB* and filamentous haemagglutinin related genes (*fhaB*, *XAC1816*) were down-regulated in both the strains (Tables 3-8 and 3-9).

In order to further understand the molecular mechanisms determining the differences in virulence and host range of Xcaw and XccA, we compared the expression profile of common genes of Xcaw and XccA. Among the 4,034 common genes, when expression of orthologous genes in Xcaw were compared to XccA, 603 genes (426 overexpressed and 177 down-regulated) in NB (Table 3-10) and 450 genes (319 overexpressed and 131 down-regulated) genes in XVM2 (Table 3-11) conditions were significantly differentially regulated at cut-off value of $\log_2$ fold change = 1.585 ($|$fold change$|$ = 3) and FDR<0.05. On comparing the differentially expressed genes in both the conditions, 126 genes were differentially regulated in Xcaw as compared to XccA irrespective of the growth conditions (Fig. 3-14). Of these, 87 were overexpressed in Xcaw and 39 genes were repressed as compared to XccA (Table 3-12). Of the 87 genes overexpressed in Xcaw, 35 were virulence-related genes including *hrpX*, *hrpG*, *phoP-phoQ* regulatory genes, TIISS substrate genes (XAC2537, XAC2763, XAC2999, XAC4004) (Table 3-12). Of the 39 genes overexpressed in XccA, 21 were virulence-

related genes including cellulase genes (XAC0028, XAC0029 and *engXCA*), reactive oxygen species (ROS)-scavenging enzyme genes e. g. superoxide dismutase gene *sodC2*, genes encoding heat shock protein GrpE and heat stress protein Muc.

Discussion

In this study, we have sequenced Xcaw, one of the variants of XccA causing citrus canker. Currently, genome sequences of XccA and Xcaw are in finished status, whereas XauB and XauC are in draft status, and XccA* is yet to be sequenced. Comparative genomic and transcriptome analyses were conducted to understand the mechanisms underlying the differences in virulence and host range of different bacterial strains.

All the citrus canker causing variants (XccA, Xcaw, XccA*, XauB, and XauC) contain PthA or its functional homologs. Thus, PthA or its functional homologs are likely one of the major pathogenicity determinant of citrus canker pathogen as suggested in a previous study by Al-Saadi et al. (2007), which linked the strains of *Xanthomonas* with different host range together. Al-Saadi et al. (2007) have shown that all the variants carry one *pthA* homolog with 17.5 repeats that determines pathogenicity on citrus and triggers immunity in various other plant species (Swarup et al. 1992). The *avrBs3*/*pthA* family of effectors includes various *pth* genes but only PthA (Swarup et al. 1992) is known to induce canker. The functional homolog of this gene in XccA strain 306 (da Silva et al. 2002) is *pthA4* which also has three other paralogs on its two plasmids (Table 3-3). We found two homologs *pthAw1* and *pthAw2* in Xcaw genome, both located on plasmid pXcaw58. The gene *pthAw2* is 99% identical to *pthA4* from XccA and also to *pthAW* sequenced from another Wellingtion strain 0053 which is able to complement a knockout mutant of *pthA* in XccA strain 3213 (Al-Saadi et al. 2007)

indicating that PthAw2 is the functional homolog of *pthA* in Xcaw. PthAw2 has the same repeat number (17.5) as other functional homologs PthA4, PthB and PthC from the three respective citrus canker causing strains XccA, XauB, and XauC (Moreira et al. 2010). The other homolog PthAw1 in Xcaw has 18.5 tandem repeats which is different from PthA homologs found in XccA that have either 15.5 or 16.5 tandem repeats. The AvrBs3/PthA family effectors are known as transcription activator-like (TAL) effectors since they reprogram host cells by specifically binding to the promoters of plant genes recognized by the central domain of tandem repeats (Boch and Bonas 2010). Comparing the DNA binding TAL effector codes for PthA from XccA as predicted by Boch and Bonas (2009) to PthAw indicate that the codes for PthA4 and PthAw2 are quite divergent (Fig. 3-6). Al-Saadi et al. (2007) predicted that the well conserved sequence of 17th repeat in functional PthA might be important for pathogenicity on citrus and this sequence is preserved in PthAw2. The rest of the sequence however encodes a DNA binding code that is only about 67% similar to the one encoded by PthA4 of XccA (Fig. 3-6). This may result in recognition of different target genes in host plant or differences in strength of induction of plant genes and thus affect virulence of Xcaw and XccA. The PthAw1 is very different from all the other sequenced PthA homologs in XccA. It remains to be investigated whether PthAw1 affects virulence in Xcaw.

Comparative analysis of Xcaw and XccA identified multiple strain-specific genes that might contribute to the differences in virulence and host range. Among the genes present in Xcaw, but absent in XccA, two effector genes, *avrGf1* and *xopAF* were identified in Xcaw, XauB and XauC but were not present in XccA genome (Table 3-3). The presence of these effectors in limited host range strains causing citrus canker and

not in the broader host range XccA makes them prime candidates for effectors that could affect host specificity. Importantly, the role of AvrGf1 in limiting the host range of Xcaw has been confirmed previously (Rybak et al. 2009). The *avrGf1*gene in Xcaw belongs to the *avrGf1* family and has been shown to trigger hypersensitive reaction in grapefruit (Rybak et al. 2009). AvrGf1 shows only about 45% identity to its homolog XAUC_04910 in XauC whereas the homolog XAUB_03570 in XauB is interrupted by a transposon and might be non-functional. When the mutant strain XcawΔ*avrGf1* was inoculated in grapefruit it caused typical canker like symptoms instead of HR, but the symptoms were visibly reduced (Rybak et al. 2009). Also, XcawΔavrGf1 does not cause disease on sweet orange (Valencia and Hamlin) as shown in Figure 3-7, indicating that there are other host limiting factors in the Xcaw genome or other virulence factors are required for XccA to overcome the plant defense and to acquire the nutrients to infect different hosts. Another candidate gene, which might contribute to host specificity, is *xopAF*, which belongs to *avrXv3* family and is located on the plasmid pXcaw58 in Xcaw. Homologs of *xopAF*, XAUB_02310 and XAUC_00300 are found in XauB and XauC but not in XccA (Table 3-3). Thus, it may contribute to restricting host range of all the three strains to limited varieties of citrus as compared to XccA. A *xopAF* homolog *avrXv3* from *X. campestris* pv. *vesicatoria* is known to induce HR in tomato line Hawaii 7981 and pepper plants (Astua-Monge et al. 2000). They also ascertained that the gene was plant inducible and regulated by the *hrp* regulatory system. The C terminal region of the protein encodes for a putative transcription activator domain indicating that it might interact with plant host genes. In this study we found that *xopAF* mutant and *xopAF avrGF1* double mutant both have lower growth *in planta* as compared to Xcaw and

avrGF1 single mutant respectively (Fig. 3-8). Though the *xopAF* mutant did not make the wellington strain pathogenic in sweet orange Valencia it slowed the growth of the pathogen in grapefruit and Mexican lime indicating that it is important for growth of the cells. Both the wellington strain and its mutants maintained their numbers in Valencia and did not die until 21 days (Fig. 3-8) indicating possibility of non-host like reaction of sweet orange to Xcaw. In addition to the effectors documented above, other effectors that differ in their presence are *xopAQ*, *xopE2*, *xopN*, *xopP* and *xopAE* present in Xcaw, XccA and XauB but not in XauC strain. Also *xopB*, *xopE4* and *xopJ1* are present in both B and C strains of Xau but missing from XccA and Xcaw. How these effectors contribute to virulence and host range of XccA, Xcaw, XauB, and XauC requires further investigation.

The LPS gene clusters of Xcaw and XccA differ (Fig. 3-9). Compared to XccA, the LPS cluster in Xcaw contains regions orthologous to both XccA and *X. oryzae* pv. *oryzicola*. The LPS is an important virulence factor that can affect host range in other Xanthomonads by protecting the bacterial cells from plant defense compounds as determined in *X. campestris* (Kingsley et al. 1993). Hence this variable LPS region of Xcaw is a potential candidate for affecting virulence and host range. LPS is also known to be a PAMP that can be recognized by plant hosts and trigger defense responses such as oxidative burst, and cell wall modifications (Dow et al. 2000; Meyer et al. 2001). Hence, the variation in LPS region as compared to XccA might have altered the recognition of Xcaw by plants thus altering its host range.

The Xcaw unique clusters also encode large numbers of phage related genes. Bacteriophages are known to facilitate horizontal gene transfer of virulence factors and

other new traits thus leading to evolution of new strains (Krylov 2003). Not surprisingly, the phage related genes showed high nucleotide identity to phage genes from *X. campestris* pv. *campestris* and *X. oryzae* pv. *oryzae*, further indicating that horizontal gene transfer has been responsible for the acquisition of these unique gene clusters. The clusters show presence of several unique transcriptional regulators (XCAW_01037, XCAW_01129, XCAW_01131, XCAW_01170) and one two-component system (TCS) sensor kinase (XCAW_01148) and its response regulator (XCAW_01150) in Xcaw that are absent in XccA. Transcriptional regulators are known to control gene expression in bacteria, whereas two-component signal transduction systems are used to modify gene expression in response to environmental conditions (Galperin 2004). These distinct regulators could be involved in regulating the gene expression of the two closely related strains differentially, thus resulting in altered virulence in host plants.

Overall, 393 genes from XccA were found to be non-orthologous to those found in Xcaw which might contribute to its broad host range of XccA. One such gene is the plant-like natriuretic peptide (PNP) encoding gene XAC2654 in XccA. XAC2654 is expressed during infection and can modify host proteome by mimicing plant hormones. It weakens host defense by affecting its photosynthetic capabilities and maintains host cellular health for better pathogen survival. Knockout of XAC2654 caused more necrosis than those observed with the wild-type, and bacterial cell death occurred earlier in the mutant. Expression of XacPNP in *X. axonopodis* pv*. vesicatoria*, caused less necrotic lesions in the host than the wild-type (Gottig et al. 2008; Garavaglia et al. 2010b). Thus, XacPNP might promote the host expansion of XccA by modifying host defense response. In addition, four genes XAC2673, XAC2903, XAC3263 and

XAC3294 that have previously been shown to be involved in virulence of XccA (Laia et al. 2009; Yan and Wang 2011) were found to be missing from Xcaw.

Many genes unique to XccA lie on its two plasmids, of which genes on the plasmid pXAC33 is mostly unique to XccA and pXAC64 has only 29 genes orthologous to genes from Xcaw. In addition to *pthA* and its functional homologs discussed in detail above, XccA contains *pthA1*, *pthA2*, and *pthA3* which are absent in Xcaw. Interestingly, *pthA1* and *pthA2* were induced in XVM2 compared to NB (Table 3-8). It has been suggested that those three PthA variants PthA1, PthA2, and PthA3 might interact with distinct host targets and contribute to virulence of XccA (Domingues et al. 2010). The other important set of genes unique to XccA is the Type IV secretion system (TIVSS). XccA contains two TIVSS clusters, the one on chromosome is homologous to the one found in Xcaw, however the one located on plasmid is completely missing from the Xcaw genome (Fig. 3-5). Different TIVSS have been found in many pathogenic bacteria with different functional characteristics (Backert and Meyer 2006). Alegria et al. (2005) identified protein-protein interactions of TIVSS proteins in XccA, where plasmid cluster appears to help in plasmid mobility and the function of the TIVSS cluster on the chromosome is unclear. The TIVSS proteins from plasmid have been shown to interact with other proteins on both plasmids as well as chromosome (Alegria et al. 2005). Furthermore, TIVSS has been shown to contribute to virulence in *X. campestris* pv. *campestris* strain 8004 (Qian et al. 2005). Thus, absence of TIVSS region might contribute to the differences in virulence and host range of Xcaw as compared to XccA.

Besides the differences in gene content, dramatic differences were observed in gene expression between Xcaw and XccA which might also contribute to the differences

in virulence and host range of the two pathogens. We studied the expression profile of XccA and Xcaw strains in XVM2 as compared to NB. At the cut-off of $\log_2$ fold change = 1.585 (|fold change| = 3) and FDR<0.05, 292 genes showed differential expression (173 up-regulated and 119 down-regulated in XVM2 compared to NB) in XccA (Table 3-8). Among them, 59 virulence related genes were induced in XVM2 compared to NB. In addition, 281 genes (129 up-regulated and 152 down-regulated in XVM2 compared to NB) were observed for Xcaw (Table 3-9). Among them, 40 virulence related genes were induced in XVM2 compared to NB. The differences in gene expression between Xcaw and XccA probably results from the differences in regulators and promoter sequences (data not shown).

The induction of the virulence genes in XVM2 condition compared to nutrient rich NB is supported by previous study (Astua-Monge et al. 2005). In the previous study, only 279 genes of XccA potentially associated with pathogenicity and virulence were tested and 31 genes were up-regulated in XVM2, while only 7 genes were repressed. In our study, we further expanded the previous study by including all genes of XccA and provide a comprehensive picture of *Xanthomonas* gene regulation. The detailed expression profile of virulence genes of Xcaw and XccA under nutrient rich (NB) and plant intercellular space mimicing (XVM2) conditions is described below.

The entire TTSS cluster consisting of 24 genes was up-regulated in XVM2 for both XccA and Xcaw strains ascertaining that XVM2 is an excellent *hrp* inducing medium. This is consistent with previous report that *Xanthomonas hrp* genes were induced in XVM2 (Schulte and Bonas 1992; Astua-Monge et al. 2005). However, only eight *hrp* genes of XccA were reported to be upregulated by XVM2 in the previous study

(Astua-Monge et al. 2005) compared to 24 induced *hrp* genes identified in this study. Among all the effectors, 16 were induced for XccA whereas 19 effectors were overexpressed for Xcaw in XVM2. In the previous study (Astua-Monge et al. 2005), only three effector genes, *avrXacE1*, *avrXacE2*, and *Xac0076* were induced in XVM2. Thus, our study further expanded the knowledge of expression of the *hrp* and effector genes in plant intercellular space mimicing (XVM2) condition.

In order to further understand the molecular mechanisms determining the differences in virulence and host range of Xcaw and XccA, we compare the expression profile of common virulence genes of Xcaw and XccA. Interestingly, both *hrpX* and *hrpG* genes were overexpressed in the Xcaw compared to XccA (Table 3-12). Both genes have been shown to be critical for virulence in *Xanthomonas* spp. (Wengelnik and Bonas 1996b). The *hrpX* gene encodes an AraC-type transcriptional activator and *hrpG* gene encodes an OmpR family regulator, both of which, are known to regulate many virulence related genes including TTSS, effector, TIISS substrate, flagella, and chemotaxis genes (Guo et al. 2011). Overexpression of Xcaw *hrpG* in *X. perforans* elicited HR in grapefruit and Mexican lime leaves by inducing *xopA* and other avirulence genes (Rybak et al. 2009). The *xopA* gene encodes harpin and was suggested to be a host-limiting factor by inducing HR. Its homologues *hpaG* and *hrpN* are also known to induce HR. The promoter regions of the *xopA* genes of Xcaw and XccA are different. However, the *xopA* gene was not overexpressed significantly in Xcaw compared to XccA (Table 3-13). The fold change of *xopA* was more than 2, but the FDR did not pass the cut off value. Five other effector genes *xopL*, *xopX*, *xopAD*, *hrpW*, and *xopAQ* were overexpressed in Xcaw in XVM2, whereas only one effector gene *xopAP* was

induced in XccA in NB (Table 3-13). Overexpression of those effector genes in Xcaw might contribute to the limited host range of Xcaw. In addition, the *phoP-phoQ* two component system genes were overexpressed in Xcaw compared to XccA (Table 3-12). The *phoP* gene encoding a response regulator is predicted to interact with various signal sensor proteins in addition to PhoQ. It is known to activate the response regulator *hrpG* in *X. oryzae* pv. *oryzae* and thus lead to a chain reaction involving activation of various virulence and growth factor genes downstream (Lee et al. 2008). The *phoQ* gene on the other hand is required for the activity of AvrXA21 in *X. oryzae* pv. *oryzae*, which determines host-variation of the strain against some rice lines (Lee et al. 2008). Thus in Xcaw, overexpression of *phoP-phoQ* could contribute to activation of certain effector genes mentioned above.

TIISS is the major protein secretion system, which secretes toxins and various degradative enzymes to breakdown the cell wall in plant hosts (Büttner and Bonas 2010). TIISS and its substrates have been shown to be important for the virulence of XccA (Yan and Wang 2012). The *xps* genes were down-regulated in XVM2 as compared to NB for Xcaw with *xpsE* being the most significantly down-regulated with $\log_2$ fold change -1.07 at FDR = 0.03. XpsE is known to be a key component of T2SS, the loss of which leads to lower virulence in *X. oryzae* (Sun et al. 2004). For XccA, the *xps* genes were not down-regulated. Down-regulation of *xps* genes in Xcaw but not in XccA might contribute to differences in virulence on different hosts of Xcaw and XccA. In XccA at least 22 genes encoding TIISS substrates were overexpressed as compared to only 12 in Xcaw. On the contrary 11 genes for Xcaw and 8 for XccA were down-regulated. Similarly, genes encoding TIISS substrates were found either down-

regulated or up-regulated (Astua-Monge et al. 2005). Specifically, four TIISS substrate protease genes (XAC2537, XAC2763, XAC2999, and XAC4004) were upregulated in Xcaw compared to XccA in both conditions (Table 3-12). Consequently, Xcaw showed higher protease activity than XccA (Fig. 3-15A). In contrast, multiple cellulase genes (XAC0028, XAC0029, and *engXCA*) were down-regulated in Xcaw compared to XccA (Table 3-12). Pectate lyase gene *pel* (XAC03562) was also down-regulated in Xcaw compared to XccA in NB medium (Table 3-10). Consequently, Xcaw showed lower pectate lyase activity as compared to XccA (Fig. 3-15B). The differential regulation of genes encoding TIISS substrates in XVM2 probably results from the different involvement of the TIISS substrates in the infection process of *Xanthomonas*. Collectively, the differences in expression of genes encoding TIISS and its substrates might contribute to the differences in virulence on different hosts of Xcaw and XccA.

Compared to Xcaw, multiple virulence genes were overexpressed in XccA which might contribute to its adaption to a broad host range (Table 3-12). These include many reactive oxygen species (ROS)-scavenging enzyme genes, e. g. superoxide dismutase gene *sodC2*, genes encoding heat shock protein GrpE and heat stress protein Muc, which indicates that XccA might be more adapted to stressful conditions due to the host defense responses of different hosts. Attachment of *Xanthomonas* to plant cell surfaces is important for pathogenicity (Rigano et al. 20007; Li and Wang 2011). Multiple genes involved in adherence were overexpressed in XccA in NB medium (Table 3-10) including filamentous haemagglutinin gene *fhaB*, *gum* genes (*gumB* to *gumK*, *gumM*), chemotaxis genes (XAC0611, XAC1666, XAC1891, XAC1893, XAC1894, XAC1895, XAC1896, XAC1897, XAC1899, XAC1900, XAC1902), *mcp* genes (XAC1996,

XAC2448, XAC2866, XAC3132), *cheA* (XAC2865), *cheR* (XAC1890), *cheR* (XAC2869), *cheY* (XAC1904) and *cheD* (XAC1889). These genes are involved in adhesion and biofilm formation of Xcaw in glass tubes as compared to XccA (Li and Wang 2011). Multiple transporter genes which are known to play critical roles for bacteria to acquire nutrients from the intercellular environment were overexpressed in XccA in XVM2 as compared to Xcaw, e.g. the potassium transporter genes *kdpB*, *kdpC* and *kdpD* and the iron siderophore transporter gene *fhuA* (XAC2185) and XAC2830 (Table 3-11). Altogether, they might contribute to the virulence on broad host of XccA as compared to Xcaw.

In conclusion, we have successfully sequenced the genome of *X. citri* subsp. *citri* strain A^w12879. Comparative genomic analysis of Xcaw and XccA indicates that Xcaw strain specific effectors XopAG and XopAF might contribute to its limited host range compared to XccA. In addition, the overexpression of avirulence/effector genes in Xcaw might also contribute to its limited host range. The overexpression of genes involved in cell wall degradation, attachment, ROS scavenging, nutrient transportation in XccA might contribute to its expansion of host range. The differential expression of genes encoding TIISS and its substrates might contribute to the differences in virulence and host range of Xcaw and XccA. Our data also demonstrate that virulence genes including genes encoding TTSS and its effectors are induced in the condition mimicing the plant intercellular environment. This study lays foundation to further characterize the mechanisms for virulence and host range of strains of *X. citri* subsp*. citri* and other bacterial pathogens.

Table 3-1. Overview of sequence data for the genome of *Xanthomonas citri* subsp. *citri* A^w 12879

Sequencing method	454 sequencing	Illumina/Solexa
Total reads	620,233	37,467,584
Total sequence output	129,503,865 bp	2,772,601,216 bp
Average read length	240 bp	74 bp
Genome coverage	24X	410X
No. of contigs[a]	378	1426

Note: 454 contigs assembled using Newbler 2.0 and Illumina/Solexa contigs assembled using CLCbio Genomics Workbench 5.0

Table 3-2. Primers used in this study

Primer	Sequence 5' → 3'
For Mutant construction	
xopAFF1	CGAATCCGAAAAGGCCAT
xopAFF2	GAggatccATTATTACACAGGCGAACG
xopAFR	AAGTAGTCGTCTCTGAAAGA
For qRT-PCR	
gnlF	TGGATAAATCGCCGGTCAAGGAGT
gnlR	ATCGGAGTTGGAGACGTACAAGGT
hrpGF	ATCGTGCTTGGACGTTTCGATTGC
hrpGR	ATTGAAAGGCAGCGCAAGGACTTC
hrpXF	AAGCGTTACTGCTCTACAACCGCT
hrpXR	TGCGCATTGGTGATCATGTAGCTG
nuoMF	ACAGGACGACATGAAGAAGCTGGT
nuoMR	ACGAAACCGTGCGAAATCATCTGC
phoPF	CTTGCGCGATGAAGGCAAGAAGTT
phoPR	ACGTGGAACGGCTTGACCAGATAA
sodC2F	AAGGGTAATGACGTCAAAGGCACG
sodC2R	ATATTGCCGTGATCGGACTGGGA
grpEF	GCCTGGACATGACCTACAAGCAAT
grpER	TTCTGGAACACCTGCACCACAT
eglF	ACTACGCCAAGTATTACGGCCACA
eglR	AGGCTCATTCATCAGCCCGAAGAT
16sF	AACGCGAAGAACCTTACCTGGTCT
16sR	TGCGGGACTTAACCCAACATCTCA

Table 3-3. General features of *Xanthomonas citri* subsp. *citri* A^w 12879 genome

	Chromosome	Plasmids	
Features	Xcaw	pXcaw19	pXcaw58
Size (bp)	5,321,499	18,869	58,317
GC content (%)	64.71	63.07	61.85
Predicted CDS			
Protein coding genes	4675	17	69
with COGs	3423	9	25
with Pfam	3552	10	32
with TIGRfam	1377	2	4
connected to KEGG pathways	1194	2	1
Ribosomal RNA	6	0	0
rRNA operons	2	0	0
Transfer RNA	54	0	0

Table 3-4. Effector repertoire of *X. citri* subsp. *citri* A^{w} 12879 (Xcaw), *X. citri* subsp. *citri* str. 306 (XccA), *X. fuscans* subsp. *aurantifolii* str. ICPB 11122 (XauB) and *X. fuscans* subsp. *aurantifolii* str. ICPB 10535 (XauC)

Effector class	Xcaw	XccA	XauB	XauC	Pfam domains	References
AvrBs2	XCAW_00465	XAC0076	XAUB_16770	XAUC_23650	Glycerophosphoryl diester phosphodiesterase	(Kearney and Staskawicz 1990)
PthA (AvrBs3, TAL)	XCAW_b00018 (PthAw1) XCAW_b00026 (pthAw2)	XACa0022 (PthA1) XACa0039 (PthA2) XACb0015 (PthA3) XACb0065 (PthA4)	XAUB_40130 XAUB_28490	XAUC_22430 XAUC_24060 XAUC_09900 XAUC_43080	Transcriptional activator, nuclear localization	(Algeria et al. 2005)
XopA (Hpa1/HpaG)	XCAW_00826	XAC0416	XAUB_19280	XAUC_43660	-	(Noel et al. 2002)
XopE1 (AvrXacE1)	XCAW_00686	XAC0286	XAUB_37010	XAUC_37580	Putative transglutaminase	(Thieme et al. 2007)
XopE3 (AvrXacE2)	XCAW_03515	XAC3224	XAUB_14680	XAUC_00040	Putative transglutaminase	(Nimchuk et al. 2007)
XopF2	XCAW_01388Ψ	XAC2785 Ψ	XAUB_07540Ψ	XAUC_21000Ψ	-	(Roden et al. 2004)
XopI	XCAW_03828	XAC0754	XAUB_39080	XAUC_07100	F-box protein	(Thieme 2008)
XopK	XCAW_03372	XAC3085	XAUB_34090	XAUC_12520	-	(Furutani et al. 2009)
XopL	XCAW_03376	XAC3090	XAUB_34130	XAUC_02900/ XAUC_12488Ψ	LRR protein	(Jiang 2007)
XopQ	XCAW_04706	XAC4333	XAUB_10220	XAUC_14670	Inosine uridine nucleoside N-ribohydrolase	(Roden et al. 2004)
XopR	XCAW_00677	XAC0277	XAUB_36920	XAUC_37490	-	(Furutani et al. 2009)
XopV	XCAW_03980	XAC0601	XAUB_23140	XAUC_21260	-	(Furutani et al. 2009)

Table 3-4. Continued.

Effector class	Xcaw	XccA	XauB	XauC	Pfam domains	References
XopX	XCAW_00956	XAC0543	XAUB_14760	XAUC_20690	-	(Metz et al. 2005)
XopZ1	XCAW_01815	XAC2009	XAUB_11532/ XAUB_13710Ψ	XAUC_25915	-	(Furutani et al. 2009)
XopAD	XCAW_00082	XAC4213	XAUB_02510	XAUC_34870	SKWP repeat protein	(Guidot et al. 2007, Petnicki-Ocwieja et al. 2002)
XopAI	XCAW_01099	XAC3230	XAUB_26830	XAUC_23780	Putative ADP-ribosyltransferase	(Thieme et al. 2005)
XopAK	XCAW_04369	XAC3666	XAUB_02580	XAUC_32490	-	(Petnicki-Ocwieja et al. 2002)
XopAP	XCAW_03269	XAC2990	XAUB_13980	XAUC_08760	-	(Mukaihara et al. 2010)
HpaA	XCAW_00810	XAC0400	XAUB_19430	XAUC_19990	T3S control protein	(Lorenz et al. 2008)
HrpW (PopW)	XCAW_03200	XAC2922	XAUB_19460	XAUC_20020	Pectate Lyase	(Park et al. 2006)
XopAQ	XCAW_03514	No annotation between XAC3223 and XAC3224	No annotation between XAUB_14670 and XAUB_14680	-	-	(Mukaihara et al. 2010)
XopE2 (AvrXacE3, AvrXccE1)	XCAW_03520	XACb0011	XAUB_31660	-	Putative transglutaminase	(Thieme et al. 2007)
XopN	XCAW_01387	XAC2786	XAUB_07520	-	ARM/HEAT repeat	(Kim et al. 2009)
XopP	XCAW_01310	XAC1208	XAUB_06720	-	-	(Roden et al. 2004)

Table 3-4. Continued.

Effector class	Xcaw	XccA	XauB	XauC	Pfam domains	References
XopAE (HpaF/HpaG)	XCAW_00801	XAC0393	XAUB_19500	-	LRR protein	(White et al. 2009)
XopC2	XCAW_01311Ψ	XAC1209Ψ XAC1210Ψ	-	-	Haloacid dehalogenase-like hydrolase	(White et al. 2009)
XopAF (AvrXv3)	XCAW_b00003	-	XAUB_02310	XAUC_00300	-	(Astua-Monge et al. 2000)
XopAG (AvrGf1/ AvrGf2)	XCAW_00608	-	XAUB_03570 Ψ	XAUC_04910	-	(Rybak et al. 2009)
XopF1 (Hpa4)	XCAW_00804/ XCAW_00805 Ψ	-	-	XAUC_31730Ψ	-	(Roden et al. 2004)
XopB	-	-	XAUB_09070/ XAUB_14842 Ψ	XAUC_00260	-	(Noel et al. 2001)
XopE4	-	-	XAUB_23330	XAUC_31730	Putative transglutaminase	(Moreira et al. 2010)
XopJ1	-	-	XAUB_20830	XAUC_08850	C55-family cysteine protease or Ser/Thr acetyltransferase	(Roden et al. 2004)

Ψ Inactive/Pseudogene

Table 3-5. Genes unique to Xcaw clustered in groups

Cluster number	Locus tag	Homologs in other genomes	Function
1	XCAW_01029 to XCAW_01071		hypothetical proteins, RhsA family protein, transcriptional regulator, integrase, adenine specific DNA methylase, type III restriction enzyme: res subunit, ATP dependent exoDNAse, thermonulease
2	XCAW_01118 to XCAW_01171	Some present in *X*. *campestris* pv. *campestris* ATCC 33913	transcriptional regulator, phage-related tail proteins, TCS response sensor and regulator, chitinase, Zn peptidase, polymerase V, transcriptional repressor, protein-glutamate methylesterase
3	XCAW_01571 to XCAW_01582	Some present in *X*. *campestris* pv. *campestris* str. 8004	phage related proteins, hypothetical protein
4	XCAW_01620 to XCAW_01631	Some present in *Acidovorax* sp. JS42	transposases, hypothetical protein, type II restriction enzyme: methylase subunit
5	XCAW_01642 to XCAW_01650	Homologous to *X*. *campestris* pv. *campestris* str. 8004	phage related regulatory proteins, chromosome partitioning related protein, hypothetical protein
6	XCAW_01654 to XCAW_01687	Homologous to *X*. *campestris* pv. *campestris* str. 8004	hypothetical proteins, soluble lytic murein transglycosylase
7	XCAW_01691 to XCAW_01719	Homologous to *X*. *campestris* pv. *campestris* str. 8004	VirB6 protein, transposases, hypothetical proteins
8	XCAW_04295 to XCAW_04303	Homologous to *X*. *oryzae* pv. *oryzicola* BLS256	lipopolysaccharide biosynthesis genes
9	XCAW_04518 to XCAW_04544	Homologous to *X*. *oryzae* pv. *oryzae* PXO99A	phage related proteins, transcriptional regulator, transposases, hypothetical proteins

Table 3-6. Summary of cDNA samples sequenced for RNA-Seq

Sample	No. of Reads	No. of reads after trim	Avg. read length after trim, bp	No. of uniquely mapped reads	No. of uniquely mapped bps x 10^8	Average coverage	mRNA reads % of uniquely mapped reads
ANB1	61,102,324	59,054,234	63.8	10,900,571	6.96	147X	18.46%
ANB2	34,861,658	34,502,250	71.0	8,505,214	6.04	129X	24.65%
ANB3	55,909,444	54,311,690	65.5	12,850,535	8.42	179X	23.66%
AXVM1	59,932,224	57,731,460	64.7	7,002,216	4.53	96X	12.13%
AXVM2	60,656,960	58,303,398	62.3	8,453,622	5.27	109X	14.50%
AXVM3	59,906,612	58,437,562	65.8	8,721,747	5.74	120X	14.93%
WNB1	64,399,896	62,871,274	68.3	14,093,510	9.63	202X	22.42%
WNB2	23,499,508	23,341,184	71.9	6,671,456	4.80	101X	28.58%
WNB3	52,110,418	48,359,192	43.0	8,185,467	3.52	77X	16.93%
WXVM1	59,681,564	58,050,261	65.5	7,998,317	5.24	111X	13.78%
WXVM2	67,385,040	62,470,162	61.5	7,068,123	4.35	95X	11.31%
WXVM3	60,841,200	51,076,418	52.0	6,561,478	3.41	79X	12.85%

Table 3-7. Degree of agreement between biological replicates for RNA-Seq

Sample	Repeats compared	R^2 value correlation coefficient
XccA in NB	ANB1 and ANB2	0.99673907
	ANB2 and ANB3	0.996187926
	ANB3 and ANB1	0.996890541
XccA inXVM2	AXVM1 and AXVM2	0.999881083
	AXVM2 and AXVM3	0.999844115
	AXVM3 and AXVM1	0.999722292
Xcaw in NB	WNB1 and WNB2	0.999145975
	WNB2 and WNB3	0.999762336
	WNB3 and WNB1	0.999658312
Xcaw in XVM2	WXVM1 and WXVM2	0.999227967
	WXVM2 and WXVM3	0.999358198
	WXVM3 and WXVM1	0.999976985

Table 3-8. Genes differentially expressed in *X. citri* subsp. *citri* str. 306 (A) in XVM2 medium (hrp inducing) as compared to NB (nutrient rich condition). Cut off value of Log2 fold Change AXVM/ANB ≥ 1.585 for overexpressed genes and ≤ -1.585 for underexpressed genes. Log2 fold change 1.585 (|fold change| 3)

Locus tag	Gene Name	Product	Log2 Fold Change AXVM/ANB
Overexpressed Genes			
XACa0031	XACa0031	transposase	91.4244951
XAC3181	lysA	diaminopimelate decarboxylase	53.73516607
XAC2259	XAC2259	hypothetical protein	48.47324504
XACb0072	XACb0072	resolvase	13.97339668
XAC0402	hrcR	type III secretion system protein	11.17800952
XAC1673	XAC1673	hypothetical protein	9.025178375
XAC3177	XAC3177	hypothetical protein	8.963896246
XAC3226	XAC3226	Tn5044 transposase	8.583043094
XAC2249	XAC2249	hypothetical protein	7.104496978
XAC3180	iucA	iron transporter	7.064570216
XAC0403	hrcQ	HrcQ protein	6.540008483
XAC3548	xadA	hypothetical protein	5.871344066
XAC3690	XAC3690	hypothetical protein	4.974578776
XAC4256	cirA	TonB-dependent receptor	4.964583959
XACb0026	XACb0026	hypothetical protein	4.96159489
XAC2653	S	phage-related tail protein	4.803492842
XAC3489	fyuA	TonB-dependent receptor	4.734157783
XAC2280	XAC2280	hypothetical protein	4.713497615
XAC0406	hrcU	type III secretion system protein HrcU	4.520513651
XAC0416	hpa1	Hpa1 protein	4.035690704
XAC0825	XAC0825	hypothetical protein	4.009380091
XAC2172	XAC2172	NADH dehydrogenase	3.634554453
XAC4338	XAC4338	hypothetical protein	3.554860845
XAC4007	XAC4007	hypothetical protein	3.417379518
XAC0753	XAC0753	hypothetical protein	3.364728257
XAC0398	hrpD6	HrpD6 protein	3.352499507
XAC1009	XAC1009	hypothetical protein	3.215722675
XAC4157	fldW	4-oxalomesaconate hydratase	3.140707774
XAC0823	phuR	outer membrane hemin receptor	3.051667562
XAC3680	XAC3680	hypothetical protein	3.041662946
XAC3490	XAC3490	amylosucrase or alpha amylase	3.010433641
XAC0758	kdpC	potassium-transporting ATPase subunit C	2.933171867
XAC0754	XAC0754	hypothetical protein	2.916595239
XAC0337	kdgT	2-keto-3-deoxygluconate permease	2.849020751
XAC0257	aceA	isocitrate lyase	2.84266408
XAC0400	hpaA	HpaA protein	2.738106242

Table 3-8. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change AXVM/ANB
XAC0255	rbcR	transcriptional regulator	2.710976372
XAC0338	XAC0338	hypothetical protein	2.703694796
XAC1766	dgoA	2-dehydro-3-deoxy-6-phosphogalactonate aldolase	2.672151326
XAC2219	XAC2219	hypothetical protein	2.639008605
XAC0822	XAC0822	hypothetical protein	2.599756547
XAC2982	qxtB	quinol oxidase subunit II	2.495353682
XAC0328	smeB	multidrug efflux transporter	2.457299532
XAC1681	XAC1681	hypothetical protein	2.451022814
XAC1576	pstC	ABC transporter phosphate permease	2.430225279
XAC3777	XAC3777	hypothetical protein	2.410883587
XAC2983	XAC2983	quinol oxidase subunit I	2.393931836
XAC1792	phoX	alkaline phosphatase	2.37667334
XACa0009	XACa0009	ISxac3 transposase	2.331692412
XAC3843	XAC3843	hypothetical protein	2.327542167
XAC3757	XAC3757	hypothetical protein	2.319496966
XAC4303	slyA	cryptic hemolysin transcriptional regulator	2.312159867
XAC2267	XAC2267	hypothetical protein	2.274095104
XAC4356	XAC4356	hypothetical protein	2.26803207
XAC3453	ilvM	acetolactate synthase isozyme II small subunit	2.263948546
XAC2853	XAC2853	cysteine protease	2.263084895
XAC0757	kdpB	potassium-transporting ATPase subunit B	2.257681733
XAC4194	XAC4194	hypothetical protein	2.226616628
XAC0334	sflA	NADH-dependent FMN reductase	2.217978209
XAC1682	rpoE	RNA polymerase sigma-E factor	2.210265862
XAC1575	pstA	ABC transporter phosphate permease	2.19717434
XAC4252	xynB	xylanase	2.185687507
XAC4225	xylA	xylose isomerase	2.177610014
XAC2838	XAC2838	LysR family transcriptional regulator	2.175983417
XAC3204	XAC3204	hypothetical protein	2.170078675
XAC1579	oprO	polyphosphate-selective porin O	2.164587442
XAC0545	aroG	phospho-2-dehydro-3-deoxyheptonate aldolase	2.144680178
XAC0693	fecA	TonB-dependent receptor	2.136279222
XAC4306	nodT	outer membrane efflux protein	2.081398082
XAC0399	hrpD5	HrpD5 protein	2.080275393
XAC0310	vanB	vanillate O-demethylase	2.067620126
XAC0364	gctA	glutaconate CoA transferase subunit A	2.065093914
XAC1873	XAC1873	hypothetical protein	2.061601373
XAC2844	mexA	multidrug resistance protein	2.056792203

Table 3-8. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change AXVM/ANB
XAC1197	XAC1197	hypothetical protein	2.038960886
XAC3497	XAC3497	hypothetical protein	2.035065213
XAC0256	mls	malate synthase	2.014744175
XAC0756	kdpA	potassium-transporting ATPase subunit A	2.01029461
XAC2202	hlyB	hemolysin secretion protein B	1.996046182
XAC4254	xynB	xylanase	1.986792522
XAC3498	fhuE	ferric iron uptake outer membrane protein	1.982667307
XAC2364	eutP	ethanolamin permease	1.981577825
XAC0899	XAC0899	hypothetical protein	1.970885848
XAC0335	XAC0335	hypothetical protein	1.969646672
XAC2218	XAC2218	hypothetical protein	1.950096088
XACa0030	XACa0030	transposase	1.942874024
XAC4064	ftrA	transcriptional activator FtrA	1.9425816
XAC2163	XAC2163	hypothetical protein	1.908393867
XAC0162	dctP	C4-dicarboxylate transport system	1.907790828
XAC2143	XAC2143	hypothetical protein	1.906583575
XAC1577	pstS	phosphate ABC transporter substrate-binding protein	1.896343446
XAC0336	metE	5-methyltetrahydropteroyltriglutamate--homocysteine S-methyltransferase	1.894636436
XAC0537	XAC0537	hypothetical protein	1.892799099
XAC2164	XAC2164	hypothetical protein	1.886338219
XAC4155	fldZ	hypothetical protein	1.883749096
XAC0737	XAC0737	transcriptional regulator	1.878535144
XAC2515	XAC2515	AsnC family transcriptional regulator	1.878202166
XAC2949	XAC2949	calcium-binding protein	1.875482776
XAC1679	ccmC	cytochrome C-type biogenesis protein	1.865566378
XAC2245	XAC2245	hypothetical protein	1.862058453
XAC3338	XAC3338	hypothetical protein	1.857715429
XAC4253	XAC4253	hypothetical protein	1.841445518
XAC3459	XAC3459	LysR family transcriptional regulator	1.841171539
XAC3692	XAC3692	hypothetical protein	1.836521986
XAC0413	hrpB7	HrpB7 protein	1.827779479
XAC0560	mdcA	malonate decarboxylase subunit alpha	1.825851893
XAC4368	fecA	TonB-dependent receptor	1.824073349
XAC1790	XAC1790	hypothetical protein	1.820176779
XAC2786	XAC2786	hypothetical protein	1.817216578
XAC1765	dgoA	galactonate dehydratase	1.803114779
XAC1433	asnB	asparagine synthetase B	1.802702416
XAC0037	XAC0037	penicillin acylase	1.796506774
XAC2399	htpX	heat shock protein HtpX	1.787884005

Table 3-8. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change AXVM/ANB
XAC1136	prpR	propionate catabolism regulatory protein	1.784579084
XAC2774	XAC2774	TonB-like protein	1.780544218
XAC0311	vanA	vanillate O-demethylase oxygenase	1.774853793
XAC3769	nucA	endonuclease	1.774624759
XAC3222	XAC3222	hypothetical protein	1.761406582
XAC3737	XAC3737	hypothetical protein	1.757594224
XAC2274	XAC2274	hypothetical protein	1.750527464
XAC2113	XAC2113	hypothetical protein	1.747782429
XAC0543	XAC0543	hypothetical protein	1.747726474
XAC0916	XAC0916	hydrolase	1.743077247
XAC1574	pstB	phosphate transporter ATP-binding protein	1.743016923
XAC1023	fecA	TonB-dependent receptor	1.742769966
XAC3085	XAC3085	hypothetical protein	1.741946347
XAC2248	XAC2248	hypothetical protein	1.730250938
XAC3552	XAC3552	hypothetical protein	1.728086337
XAC4305	fusE	fusaric acid resistance protein	1.721852333
XAC0396	hpaB	HpaB protein	1.720831425
XAC4062	fhuA	TonB-dependent receptor	1.710547693
XAC3488	suc1	sugar transporter	1.70804397
XAC2285	orf84	hypothetical protein	1.705264507
XAC3053	XAC3053	hypothetical protein	1.700863999
XAC4350	XAC4350	transcriptional regulator	1.699812386
XAC0297	XAC0297	hypothetical protein	1.694139167
XAC3954	XAC3954	hypothetical protein	1.691303605
XAC1927	aslB	Fe-S oxidoreductase	1.68552461
XAC4192	XAC4192	hypothetical protein	1.684456718
XAC3176	fecA	citrate-dependent iron transporter	1.682395955
XAC0074	cirA	TonB-dependent receptor	1.679957329
XAC4227	aguA	alpha-glucuronidase	1.673235652
XAC0363	vanA	vanillate O-demethylase oxygenase	1.672624497
XAC1196	lexA	LexA repressor	1.672059517
XAC1770	celA	cellulase	1.66986911
XAC2763	XAC2763	extracellular protease	1.668484952
XAC1702	XAC1702	Mg-protoporphyrin IX monomethyl ester oxidative cyclase	1.66610193
XAC2626	fimT	fimbrial biogenesis protein	1.661546548
XAC3984	XAC3984	hypothetical protein	1.658630042
XAC0492	XAC0492	bacterioferritin-associated ferredoxin	1.656523625
XAC2243	orf8	plasmid-like protein	1.6549515
XAC0202	XAC0202	hypothetical protein	1.653190711

Table 3-8. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change AXVM/ANB
XAC2746	XAC2746	metallopeptidase	1.651225818
XAC0414	hrcT	HrcT protein	1.645206342
XAC2212	topB	DNA topoisomerase III	1.63848051
XAC1820	thrA	bifunctional aspartokinase I/homoserine dehydrogenase I	1.636258424
XAC1680	XAC1680	serine protease	1.636225787
XAC3747	ybdR	Zn-dependent alcohol dehydrogenase	1.630208116
XAC0878	pcaH	protocatechuate 4,5-dioxygenase subunit beta	1.626181497
XAC1651	XAC1651	TonB-like protein	1.625943134
XAC1181	XAC1181	hypothetical protein	1.614724598
XAC2843	mexB	multidrug efflux transporter	1.614288819
XAC3866	XAC3866	hypothetical protein	1.61071669
XAC4138	XAC4138	transposase	1.608738524
XAC1685	XAC1685	cytochrome C	1.608524014
XAC0612	engXCA	cellulase	1.607513009
XAC2561	blc	outer membrane lipoprotein Blc	1.605041752
XAC1161	XAC1161	hypothetical protein	1.597790636
XAC0163	dctQ	C4-dicarboxylate membrane transport protein	1.596821527
XAC1789	XAC1789	hypothetical protein	1.589554846
XAC1578	phoX	phosphate-binding protein	1.589114245
XAC0917	XAC0917	transcriptional regulator	1.588678732
XAC1160	XAC1160	oxidoreductase	1.587648214
Underexpressed Genes			
XAC3178	XAC3178	hypothetical protein	-16.88666299
XAC4255	exuT	hexuranate transporter	-7.192282312
XAC0116	XAC0116	hypothetical protein	-6.973927206
XAC1814	fhaC	outer membrane hemolysin activator protein	-6.097046532
XAC2548	XAC2548	oxidoreductase	-5.982748884
XAC0824	XAC0824	hypothetical protein	-5.53161883
XACb0051	ISxac2	ISxac2 transposase	-5.419377359
XAC3754	XAC3754	hypothetical protein	-5.146227128
XAC2549	XAC2549	D-amino acid oxidase	-3.599951465
XAC3520	XAC3520	hypothetical protein	-2.896127295
XAC2539	XAC2539	hypothetical protein	-2.87796941
XAC0516	XAC0516	hypothetical protein	-2.738068666

Table 3-8. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change AXVM/ANB
XAC2546	XAC2546	ketoglutarate semialdehyde dehydrogenase	-2.678728672
XAC2868	vieA	response regulator	-2.630814242
XAC3179	yceE	transporter	-2.619277998
XAC0350	XAC0350	hypothetical protein	-2.570246784
XAC2541	XAC2541	peptidase	-2.4839614
XAC2540	XAC2540	hypothetical protein	-2.482935843
XAC2550	XAC2550	hypothetical protein	-2.47376968
XAC2545	pepQ	proline dipeptidase	-2.435703559
XAC2531	btuB	TonB-dependent receptor	-2.398041276
XAC3753	XAC3753	hypothetical protein	-2.335154867
XAC2547	dapA	dihydrodipicolinate synthetase	-2.327334203
XAC0614	XAC0614	diguanylate cyclase	-2.319954402
XAC1314	paaF	enoyl-CoA hydratase	-2.273135291
XAC2543	XAC2543	hypothetical protein	-2.227249442
XAC1901	XAC1901	hypothetical protein	-2.212326226
XAC1695	XAC1695	hypothetical protein	-2.203648497
XAC2544	XAC2544	hypothetical protein	-2.196500695
XAC0518	XAC0518	hypothetical protein	-2.192309509
XAC1803	XAC1803	hypothetical protein	-2.179683683
XAC3635	XAC3635	hypothetical protein	-2.162789585
XAC1900	tsr	chemotaxis protein	-2.162665841
XAC3294	XAC3294	hypothetical protein	-2.115589002
XAC0749	ribA	3,4-dihydroxy-2-butanone 4-phosphate synthase	-2.110168405
XAC0853	XAC0853	hypothetical protein	-2.086058433
XAC2535	btuB	TonB-dependent receptor	-2.075039441
XAC1130	trpE	hypothetical protein	-2.065691257
XAC1899	tsr	chemotaxis protein	-2.047540487
XAC0520	XAC0520	acyltransferase	-2.043733249
XAC2542	yveA	amino acid permease	-2.041106275
XAC1897	tsr	chemotaxis protein	-2.022977015
XAC1313	fadE9	acyl-CoA dehydrogenase	-2.015280259
XAC4134	XAC4134	hypothetical protein	-1.996637065
XAC3330	cysJ	NADPH-sulfite reductase flavoprotein subunit	-1.98662768
XAC1940	XAC1940	diguanylate cyclase	-1.983751862
XAC1935	flhF	flagellar biosynthesis regulator FlhF	-1.96685314
XAC1936	flhA	flagellar biosynthesis protein FlhA	-1.928453971
XAC2144	XAC2144	serine protease	-1.912143648
XAC0747	XAC0747	hypothetical protein	-1.904861098

Table 3-8. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change AXVM/ANB
XAC1315	XAC1315	enoyl-CoA hydratase	-1.903057101
XAC3662	XAC3662	hypothetical protein	-1.895677175
XAC1890	cheR	chemotaxis protein methyltransferase	-1.895667171
XAC0748	ribE	riboflavin synthase subunit alpha	-1.895299492
XAC2375	XAC2375	hypothetical protein	-1.894255325
XAC1937	flhB	flagellar biosynthesis protein FlhB	-1.890179824
XAC2866	mcp	chemotaxis protein	-1.888602271
XAC1941	fliR	flagellar biosynthetic protein	-1.87915101
XAC0471	XAC0471	hypothetical protein	-1.871060264
XAC3444	btuB	TonB-dependent receptor	-1.851599574
XAC2156	XAC2156	hypothetical protein	-1.850920329
XAC0107	XAC0107	hypothetical protein	-1.850894531
XAC1946	fliN	flagellar protein	-1.838883742
XAC1985	flgC	flagellar basal body rod protein FlgC	-1.834499798
XAC1003	XAC1003	hypothetical protein	-1.833636126
XAC3120	glk	glucokinase	-1.831271258
XAC1891	tsr	chemotaxis protein	-1.830609851
XAC1312	mmsA	methylmalonate-semialdehyde dehydrogenase	-1.809288575
XAC4012	XAC4012	hypothetical protein	-1.808698614
XAC1948	fliL	flagellar protein	-1.802809033
XAC1942	fliQ	flagellar biosynthesis	-1.80252679
XAC1988	flgA	flagellar basal body P-ring biosynthesis protein FlgA	-1.7766389
XAC0263	accC	biotin carboxylase	-1.760031408
XAC3317	XAC3317	acetyltransferase	-1.757740341
XAC0860	oppD	ABC transporter ATP-binding protein	-1.756373443
XAC4011	XAC4011	hypothetical protein	-1.74650994
XAC2447	cheW	chemotaxis protein	-1.731056692
XAC1979	flgI	flagellar basal body P-ring biosynthesis protein FlgA	-1.7296501
XAC1896	tsr	chemotaxis protein	-1.725885815
XAC2619	virB10	VirB10 protein	-1.724072505
XAC1993	XAC1993	hypothetical protein	-1.72015485
XAC3072	fucA1	alpha-L-fucosidase	-1.718648011
XAC1816	XAC1816	hemagglutinin/hemolysin-like protein	-1.718581073
XAC4048	iroN	TonB-dependent receptor	-1.698968733
XAC1980	flgH	flagellar basal body L-ring protein	-1.694451501
XAC3121	fepA	TonB-dependent receptor	-1.685657847
XAC4149	XAC4149	hypothetical protein	-1.684285848
XAC2865	cheA	chemotaxis histidine protein kinase	-1.682014261

Table 3-8. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change AXVM/ANB
XAC0205	glnB	nitrogen regulatory protein P-II	-1.677954471
XAC0265	acdA	acyl-CoA dehydrogenase	-1.662755489
XAC2617	virB1	VirB1 protein	-1.661587297
XAC1955	fliE	flagellar protein	-1.658879997
XAC1396	XAC1396	hypothetical protein	-1.654943159
XAC2379	XAC2379	hypothetical protein	-1.654198927
XAC0611	tsr	chemotaxis protein	-1.649954054
XAC1930	cheA	chemotaxis protein	-1.648894486
XAC2065	acrD	transporter	-1.646265611
XAC1815	fhaB	filamentous hemagglutinin	-1.646120848
XAC2534	XAC2534	hypothetical protein	-1.643799724
XAC1497	XAC1497	hypothetical protein	-1.643559245
XAC1987	cheV	chemotaxis protein	-1.642179208
XAC1947	fliM	flagellar motor switch protein FliM	-1.642060203
XAC0952	pth	peptidyl-tRNA hydrolase	-1.637296939
XAC1389	yfiL	ABC transporter ATP-binding protein	-1.634240173
XAC2622	XAC2622	hypothetical protein	-1.625290485
XAC2620	virB9	VirB9 protein	-1.622636683
XAC3999	XAC3999	hypothetical protein	-1.620704973
XAC1865	recJ	single-stranded-DNA-specific exonuclease	-1.620226243
XAC3133	yggA	membrane transport protein	-1.618542941
XAC1146	fecA	TonB-dependent receptor	-1.616210473
XAC2483	XAC2483	hypothetical protein	-1.612738679
XAC1906	cheW	chemotaxis protein	-1.611391377
XAC4164	XAC4164	hypothetical protein	-1.606491793
XAC1945	fliO	flagellar protein	-1.596788766
XAC0829	XAC0829	ABC transporter substrate-binding protein	-1.594381604
XAC1954	fliF	flagellar MS-ring protein	-1.594165125
XAC2618	virB11	VirB11 protein	-1.58976968
XAC2482	rrpX	transcriptional regulator	-1.589195117
XAC1502	XAC1502	hypothetical protein	-1.585753337

Table 3-9. Genes differentially expressed in *X. citri* subsp. *citri* str. A^w 12879 (W) in XVM2 medium (hrp inducing) as compared to NB (nutrient rich condition). Cut off value of Log2 fold Change WXVM/WNB ≥ 1.585 for overexpressed genes and ≤ -1.585 for underexpressed genes. Log2 fold change 1.585 (|fold change|=3)

Locus tag	Gene Name	Product	Log_2 Fold Change WXVM/WNB
Overexpressed Genes			
XCAW_01553	czcA	silver efflux pump	86.00429147
XCAW_00810	hpaA	hpaA protein	48.8765859
XCAW_01579	XCAW_01579	phage replication protein RstA	15.15173601
XCAW_02384	wbbJ	Acetyltransferases (the isoleucine patch superfamily)	12.30102632
XCAW_01571	XCAW_01571	Hypothetical protein	9.398602024
XCAW_02450	XCAW_02450	Hypothetical Protein	8.521995782
XCAW_03444	cirA	Outer membrane receptor protein, mostly Fe transport	7.304901959
XCAW_00816	hrcU	HrcU protein	6.651595141
XCAW_00738	sflA	NADH-dependent FMN reductase	5.919992563
XCAW_01386	XCAW_01386	Hypothetical Protein	4.752781261
XCAW_00739	XCAW_00739	Hypothetical Protein	4.238760923
XCAW_02503	XCAW_02503	Hypothetical Protein	3.81974007
XCAW_00731	smeB	multidrug efflux transporter	3.634581473
XCAW_02971	XCAW_02971	Hypothetical Protein	3.41784518
XCAW_00656	aceA	Isocitrate lyase	3.366724684
XCAW_02513	mhpC	hydrolase	3.345425704
XCAW_04531	XCAW_04531	Hypothetical Protein	3.321854834
XCAW_00826	hpa1	Hap1 protein	3.284402619
XCAW_00740	metE	Methionine synthase II (cobalamin-independent)	3.152363076
XCAW_01800	cirA	Outer membrane receptor protein, mostly Fe transport	3.071973882
XCAW_01679	XCAW_01679	Hypothetical Protein	2.996833119
XCAW_02390	rpoE	RNA polymerase sigma-E factor	2.985415596

Table 3-9. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change WXVM/WNB
XCAW_00817	hrpB1	HrpB1 protein	2.957612445
XCAW_04481	XCAW_04481	Hypothetical protein	2.941814698
XCAW_03573	XCAW_03573	Hypothetical Protein	2.862291397
XCAW_00413	XCAW_00413	Hypothetical Protein	2.773789229
XCAW_00809	hrpD5	HrpD5 protein	2.715655807
XCAW_02099	tra5	transposase	2.64786895
XCAW_03134	XCAW_03134	PAP2 (acid phosphatase) superfamily protein	2.598913765
XCAW_03161	XCAW_03161	Hypothetical Protein	2.556477892
XCAW_01947	XCAW_01947	Hypothetical Protein	2.509564442
XCAW_00498	trxA	Thioredoxin	2.498029193
XCAW_01534	araC	AraC-type DNA-binding domain-containing protein	2.483440772
XCAW_00913	XCAW_00913	Hypothetical Protein	2.47503809
XCAW_01678	XCAW_01678	Hypothetical Protein	2.446636888
XCAW_04186	cirA	Outer membrane receptor protein, mostly Fe transport	2.437688807
XCAW_04216	bcsA	Glycosyltransferase probably involved in cell wall biogenesis	2.36772409
XCAW_04187	amyA	Glycosidase	2.345488068
XCAW_03339	mhpC	hydrolase	2.339989105
XCAW_00741	kdgT	2-keto-3-deoxygluconate permease	2.308173551
XCAW_02370	XCAW_02370	Fe-S oxidoreductases family 2	2.306884208
XCAW_04628	XCAW_04628	Hypothetical Protein	2.302151917
XCAW_02302	Eda	2-keto-3-deoxy-6-phosphogluconate aldolase	2.291114771
XCAW_03307	XCAW_03307	Hypothetical Protein	2.222122648
XCAW_01319	XCAW_01319	Cysteine protease	2.195405549
XCAW_04201	tra5	transposase	2.174463941
XCAW_00655	aceB	Malate synthase	2.148518656
XCAW_01723	xerC	Integrase	2.126026528
XCAW_01672	XCAW_01672	Hypothetical Protein	2.120245927
XCAW_03324	mET2	Homoserine acetyltransferase	2.119347649

Table 3-9. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change WXVM/WNB
XCAW_02038	Pel	Pectate lyase	2.105781403
XCAW_01690	radC	DNA repair protein	2.089990882
XCAW_03862	betT	Choline-glycine betaine transporter	2.084851267
XCAW_01112	XCAW_01112	Hypothetical Protein	2.082537527
XCAW_01646	XCAW_01646	Hypothetical Protein	2.069207669
XCAW_04607	XCAW_04607	Hypothetical Protein	2.064680343
XCAW_03975	Nei	Formamidopyrimidine-DNA glycosylase	2.063945516
XCAW_00654	lysR	Transcriptional regulator	2.060757909
XCAW_00806	hpaB	HpaB protein	2.060550373
XCAW_00433	XCAW_00433	Hypothetical Protein	2.033307194
XCAW_02392	aprE	Subtilisin-like serine protease	2.029176025
XCAW_00802	hrpF	HrpF protein	2.021961685
XCAW_01387	XCAW_01387	Hypothetical Protein	2.013929222
XCAW_00686	avrXacE1	avirulence protein	2.010636575
XCAW_00958	aroG	3-Deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthase	1.996777108
XCAW_01632	hepA	Superfamily II DNA	1.994982692
XCAW_03756	cirA	Outer membrane receptor protein, mostly Fe transport	1.988799152
XCAW_03665	mhpC	hydrolase	1.985100655
XCAW_03492	XCAW_03492	Hypothetical Protein	1.958283503
XCAW_01665	XCAW_01665	Hypothetical Protein	1.956543531
XCAW_01110	XCAW_01110	Hypothetical Protein	1.936806015
XCAW_04534	XCAW_04534	Hypothetical Protein	1.928305447
XCAW_01615	syrE1	ATP-dependent serine activating enzyme	1.926670915
XCAW_01649	XCAW_01649	Hypothetical Protein	1.920245314
XCAW_00685	XCAW_00685	Hypothetical Protein	1.917812498
XCAW_00528	XCAW_00528	Hypothetical Protein	1.915049604
XCAW_03135	yjdB	membrane-associated, metal-dependent hydrolase	1.907833408
XCAW_03112	XCAW_03112	Hypothetical Protein	1.887882446

Table 3-9. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change WXVM/WNB
XCAW_01271	XCAW_01271	Hypothetical Protein	1.872765196
XCAW_00521	XCAW_00521	Hypothetical Protein	1.863676335
XCAW_02910	asnB	Asparagine synthase (glutamine-hydrolyzing)	1.860877397
XCAW_04388	XCAW_04388	Hypothetical Protein	1.856936469
XCAW_03828	XCAW_03828	Hypothetical Protein	1.845481551
XCAW_01647	XCAW_01647	Hypothetical Protein	1.837950142
XCAW_00141	fldW	4-oxalomesaconate hydratase	1.833762556
XCAW_00238	ftrA	Transcriptional regulator	1.830484771
XCAW_03227	XCAW_03227	Hypothetical Protein	1.821126913
XCAW_00100	XCAW_00100	Hypothetical Protein	1.806495705
XCAW_01614	syrE2	ATP-dependent serine activating enzyme	1.783262132
XCAW_00750	XCAW_00750	Integrase	1.781219675
XCAW_02422	XCAW_02422	Hypothetical Protein	1.777655981
XCAW_00974	mdcA	malonate decarboxylase subunit alpha	1.759295729
XCAW_00497	atsE	Protein required for attachment to host cell	1.756206791
XCAW_00041	cirA	Outer membrane receptor protein, mostly Fe transport	1.755436711
XCAW_00769	Hmp	Flavodoxin reductases (ferredoxin-NADPH reductases) family 1	1.749997983
XCAW_01648	XCAW_01648	Hypothetical Protein	1.746495275
XCAW_01683	XCAW_01683	Hypothetical Protein	1.741760322
XCAW_02651	fimT	fimbrial biogenesis protein	1.741740548
XCAW_04317	XCAW_04317	Hypothetical Protein	1.735545735
XCAW_00608	avrGf1	avirulence protein	1.732104322
XCAW_02982	araJ	Arabinose efflux permease	1.714674032
XCAW_02746	XCAW_02746	Hypothetical Protein	1.711024522
XCAW_04153	lysR	Transcriptional regulator	1.706208886
XCAW_04120	cirA	Outer membrane receptor protein, mostly Fe transport	1.70382146
XCAW_01772	XCAW_01772	Oxidoreductase	1.693736061
XCAW_04055	blaI	transcriptional regulator	1.692014478

Table 3-9. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change WXVM/WNB
XCAW_00956	XCAW_00956	Hypothetical Protein	1.686005416
XCAW_03514	XCAW_03514	xanthomonas outer protein AQ	1.653748637
XCAW_02711	citB	Response regulator	1.652092283
XCAW_02991	dksA	DnaK suppressor protein	1.647424892
XCAW_04318	bioD	Dethiobiotin synthetase	1.645368497
XCAW_04426	XCAW_04426	Hypothetical Protein	1.640092478
XCAW_03037	XCAW_03037	transcriptional regulator	1.638786568
XCAW_04184	proP	Permeases of the major facilitator superfamily	1.636778001
XCAW_02192	Lrp	Transcriptional regulator	1.634101619
XCAW_01236	pspF	Transcriptional regulator	1.632378224
XCAW_01107	gp19	DNA maturase	1.623983311
XCAW_01328	acrA	Membrane-fusion protein	1.610864633
XCAW_01797	XCAW_01797	Hypothetical Protein	1.610738674
XCAW_04428	XCAW_04428	Hypothetical Protein	1.602655579
XCAW_01722	lysR	Transcriptional regulator	1.5958619
XCAW_02168	proP	Permeases of the major facilitator superfamily	1.595767988
XCAW_02745	cynT	Carbonic anhydrase	1.592269693
XCAW_04389	XCAW_04389	Hypothetical Protein	1.590899382
XCAW_00034	XCAW_00034	Hypothetical Protein	1.590614349
XCAW_00807	hrpE	HrpE protein	1.590196171
XCAW_01066	XCAW_01066	Hypothetical Protein	1.589789203
XCAW_01390	XCAW_01390	Hypothetical Protein	1.587621746
XCAW_03704	XCAW_03704	Protein involved in meta-pathway of phenol degradation	1.585067241

Table 3-9. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change WXVM/WNB
Underexpressed Genes			
XCAW_02378	XCAW_02378	Hypothetical Protein	-3140.515625
XCAW_00042	proP	Permeases of the major facilitator superfamily	-83.0267454
XCAW_00624	XCAW_00624	Hypothetical Protein	-35.15380886
XCAW_02461	XCAW_02461	Hypothetical Protein	-22.23939813
XCAW_00151	araC	AraC-type DNA-binding domain-containing protein	-19.75201875
XCAW_01627	XCAW_01627	Hypothetical Protein	-11.82025355
XCAW_02589	fhaC	Hemolysin activation	-9.636922617
XCAW_01336	araJ	Arabinose efflux permease	-9.528786753
XCAW_02227	hcaD	NAD(FAD)-dependent dehydrogenase	-9.001986184
XCAW_02229	XCAW_02229	D-amino acid oxidase	-8.796458825
XCAW_00602	glnK	Nitrogen regulatory protein PII	-7.276308634
XCAW_02376	smtA	SAM-dependent methyltransferase	-5.958742576
XCAW_00168	XCAW_00168	Hypothetical Protein	-5.278464915
XCAW_01895	flhF	Flagellar GTP-binding protein	-4.31455307
XCAW_02375	XCAW_02375	Hypothetical Protein	-4.094281955
XCAW_01883	fliN	Flagellar motor switch	-4.066845786
XCAW_02225	putA	NAD-dependent aldehyde dehydrogenase	-4.031671388
XCAW_00496	XCAW_00496	Hypothetical Protein	-3.888352375
XCAW_02221	potE	Amino acid transporter	-3.324988986
XCAW_02224	pepP	Xaa-Pro aminopeptidase	-2.973159044
XCAW_03034	paaF	Enoyl-CoA hydratase	-2.965802973
XCAW_02220	dAP2	Dipeptidyl aminopeptidase	-2.914007554
XCAW_01230	trpE	Anthranilate synthase component I	-2.851920531
XCAW_02219	lacA	Beta-galactosidase	-2.823701003
XCAW_03079	rsbW	Two-component system sensor protein	-2.708440947
XCAW_03927	acoR	Transcriptional activator of acetoin	-2.704095317

Table 3-9. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change WXVM/WNB
XCAW_02226	dapA	Dihydrodipicolinate synthase	-2.637251109
XCAW_03833	ribB	3,4-dihydroxy-2-butanone 4-phosphate synthase	-2.586237557
XCAW_02223	XCAW_02223	Hypothetical Protein	-2.585904159
XCAW_02230	XCAW_02230	Proline racemase	-2.572001105
XCAW_01884	fliO	Flagellar biosynthesis	-2.489272539
XCAW_00928	pgpB	Membrane-associated phospholipid phosphatase	-2.454176248
XCAW_02222	XCAW_02222	Hypothetical Protein	-2.452372612
XCAW_00854	aceF	Dihydrolipoamide acyltransferase	-2.447742182
XCAW_01847	flgH	Flagellar basal body L-ring protein	-2.432807524
XCAW_00603	amtB	Ammonium transporter	-2.412986746
XCAW_01848	flgI	Flagellar basal-body P-ring protein	-2.340453908
XCAW_00734	cmfA	conditioned medium factor	-2.33344351
XCAW_00110	XCAW_00110	Metal-dependent hydrolase	-2.327534492
XCAW_01343	fhuA	TonB-dependent receptor	-2.326107186
XCAW_04243	aprE	Subtilisin-like serine protease	-2.325548227
XCAW_02218	XCAW_02218	Hypothetical Protein	-2.319421627
XCAW_02213	cirA	Outer membrane receptor protein, mostly Fe transport	-2.286199995
XCAW_03834	ribC	Riboflavin synthase alpha chain	-2.268832606
XCAW_00855	XCAW_00855	Hypothetical Protein	-2.257625853
XCAW_00111	Tas	oxidoreductases (related to aryl-alcohol dehydrogenases)	-2.247298472
XCAW_03033	XCAW_03033	Enoyl-CoA hydratase	-2.230314362
XCAW_01893	flhA	Flagellar biosynthesis protein FlhA	-2.224098334
XCAW_03987	XCAW_03987	archaeal methyltransferase	-2.220823106
XCAW_03775	dAP2	Dipeptidyl aminopeptidase	-2.176404655
XCAW_00292	speB	Arginase	-2.148387728
XCAW_00927	pldB	Lysophospholipase	-2.130227195
XCAW_02252	fabH	3-oxoacyl-[acyl-carrier-protein] synthase III	-2.120390929
XCAW_02508	Tar	Methyl-accepting chemotaxis protein	-2.113973547

Table 3-9. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change WXVM/WNB
XCAW_01849	flgJ	Flagellum-specific muramidase	-2.096489665
XCAW_03359	fucA1	Alpha-L-fucosidase	-2.096467276
XCAW_03035	fadE9	Acyl-CoA dehydrogenase	-2.089155085
XCAW_03815	XCAW_03815	Methyltransferase	-2.08866952
XCAW_03032	mmsB	3-hydroxyisobutyrate dehydrogenase	-2.059001619
XCAW_01882	fliM	Flagellar motor switch protein	-2.056316691
XCAW_04041	XCAW_04041	Hypothetical Protein	-2.037612969
XCAW_03036	putA	NAD-dependent aldehyde dehydrogenase	-2.006100409
XCAW_01226	fabD	Malonyl CoA-ACP transacylase	-2.004394574
XCAW_00766	glpF	Glycerol uptake facilitator and related permeases (Major Intrinsic Protein Family)	-1.986165469
XCAW_01854	XCAW_01854	Hypothetical Protein	-1.983088897
XCAW_02550	leuA	Hydroxymethylglutaryl-CoA lyase	-1.981592481
XCAW_00150	uspA	Universal stress protein and related nucleotide-binding protein	-1.980338221
XCAW_02209	cirA	Outer membrane receptor protein, mostly Fe transport	-1.979295018
XCAW_04455	motB	Flagellar motor protein	-1.976906999
XCAW_04166	gltP	C4-dicarboxylate transport protein	-1.974478906
XCAW_00380	anmK	anhydro-N-acetylmuramic acid kinase	-1.935674172
XCAW_02611	sglT	sodium/glucose cotransport protein	-1.925477209
XCAW_01897	fliA	DNA-directed RNA polymerase specialized sigma subunit	-1.920358389
XCAW_00099	ndvB	Cellobiose phosphorylase	-1.919682419
XCAW_01786	XCAW_01786	Adenosine deaminase	-1.914461422
XCAW_01900	cheA	Chemotaxis protein histidine kinase	-1.912684979
XCAW_03381	rimL	Acetyltransferase, including N-acetylases of ribosomal protein	-1.907245017
XCAW_01996	cycH	Cytochrome c biogenesis factor	-1.898633138
XCAW_00254	cirA	Outer membrane receptor protein, mostly Fe transport	-1.885017979
XCAW_01757	XCAW_01757	Cation/multidrug efflux pump	-1.867038088
XCAW_00858	nucH	extracellular nuclease	-1.857602062

Table 3-9. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change WXVM/WNB
XCAW_01896	fleN	flagellar synthesis regulator	-1.843600925
XCAW_00856	acoB	Thiamine pyrophosphate-dependent dehydrogenase, E1 component beta subunit	-1.825311085
XCAW_01846	flgG	Flagellar basal body rod protein	-1.817249914
XCAW_03383	cheB	Chemotaxis response regulator	-1.816912793
XCAW_04726	potE	Amino acid transporter	-1.815688935
XCAW_04311	Gst	Glutathione-S-transferase	-1.812598077
XCAW_01881	fliL	Flagellar basal body-associated protein	-1.798105595
XCAW_04371	yaeE	Permease component of an uncharacterized ABC transporter	-1.791960586
XCAW_03377	cutC	Uncharacterized protein involved in copper resistance	-1.791837307
XCAW_04325	fabA	3-hydroxydecanoyl-ACP dehydratase	-1.790536413
XCAW_01304	oliA	oligopeptide transporter	-1.780547639
XCAW_03204	proC	Pyrroline-5-carboxylate reductase	-1.778101035
XCAW_01009	rimL	Acetyltransferase, including N-acetylases of ribosomal protein	-1.777243904
XCAW_00910	XCAW_00910	Hypothetical Protein	-1.763202433
XCAW_01845	flgF	Flagellar basal body rod protein	-1.762564656
XCAW_01875	fliF	Flagellar MS-ring protein	-1.752591022
XCAW_00289	XCAW_00289	Hypothetical Protein	-1.747286414
XCAW_00957	cirA	Outer membrane receptor protein, mostly Fe transport	-1.743589863
XCAW_03836	ribD	Pyrimidine reductase, riboflavin biosynthesis	-1.737362909
XCAW_00507	moxR	MoxR-like ATPase	-1.728262727
XCAW_02022	proP	Permeases of the major facilitator superfamily	-1.724564363
XCAW_02044	XCAW_02044	Hypothetical Protein	-1.720651997
XCAW_01844	flgE	Flagellar basal body and hook protein	-1.71809943
XCAW_02214	XCAW_02214	Hypothetical Protein	-1.716045903
XCAW_01850	flgK	Flagellar hook-associated protein FlgK	-1.711306816
XCAW_02062	XCAW_02062	Flavoprotein	-1.706080685
XCAW_01432	Mrp	ATPases involved in chromosome partitioning	-1.704145303

Table 3-9. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change WXVM/WNB
XCAW_02292	XCAW_02292	Hypothetical Protein	-1.700790245
XCAW_00105	fucP	Fucose permease	-1.700275909
XCAW_02602	XCAW_02602	Hypothetical Protein	-1.697828188
XCAW_04559	Sun	tRNA and rRNA cytosine-C5-methylase	-1.694698627
XCAW_04198	XCAW_04198	Hypothetical Protein	-1.690773264
XCAW_03275	XCAW_03275	Hypothetical Protein	-1.679903791
XCAW_03360	XCAW_03360	glycosyl hydrolase	-1.67691337
XCAW_04090	Rph	Ribonuclease PH	-1.670466616
XCAW_03010	ubiH	2-polyprenyl-6-methoxyphenol hydroxylase and related FAD-dependent oxidoreductase	-1.67000733
XCAW_00121	actP	acetate permease	-1.668686991
XCAW_04453	XCAW_04453	Hypothetical Protein	-1.665486282
XCAW_00403	XCAW_00403	Hypothetical Protein	-1.664782279
XCAW_01899	cheZ	Chemotaxis protein	-1.661124837
XCAW_04099	XCAW_04099	Hypothetical Protein	-1.658567365
XCAW_00615	XCAW_00615	Hypothetical Protein	-1.655623816
XCAW_02291	xylB	Sugar (pentulose and hexulose) kinase	-1.654721931
XCAW_00107	XCAW_00107	L-alanine-DL-glutamate epimerase and related enzymes of enolase superfamily	-1.654597714
XCAW_02507	XCAW_02507	Hypothetical Protein	-1.651152371
XCAW_04042	XCAW_04042	membrane protein	-1.649529349
XCAW_03406	Glk	Glucokinase	-1.649521898
XCAW_00865	hmgA	Homogentisate 1,2-dioxygenase	-1.646718087
XCAW_01421	yxaH	Hypothetical Protein	-1.641362704
XCAW_04244	xadA	Autotransporter adhesin	-1.640441319
XCAW_02061	XCAW_02061	Uncharacterized protein conserved in bacteria	-1.636697842
XCAW_01843	flgD	Flagellar hook capping protein	-1.635728587
XCAW_00147	XCAW_00147	Uncharacterized protein conserved in bacteria	-1.634511561

Table 3-9. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change WXVM/WNB
XCAW_01337	Tas	oxidoreductases (related to aryl-alcohol dehydrogenases)	-1.633424558
XCAW_03630	Pth	Peptidyl-tRNA hydrolase	-1.62687934
XCAW_02547	caiD	Enoyl-CoA hydratase	-1.626642034
XCAW_04074	pilP	Fimbrial assembly protein	-1.621647139
XCAW_00149	wbbJ	Acetyltransferase (the isoleucine patch superfamily)	-1.619862241
XCAW_03363	bglX	Beta-glucosidase-related glycosidase	-1.619597025
XCAW_00133	XCAW_00133	Uncharacterized protein conserved in bacteria	-1.618717367
XCAW_02928	cdsA	CDP-diglyceride synthetase	-1.618284133
XCAW_03384	cheA	Chemotaxis protein histidine kinase	-1.618182349
XCAW_04326	fabB	3-oxoacyl-(acyl-carrier-protein) synthase	-1.617250514
XCAW_02157	proP	Permeases of the major facilitator superfamily	-1.614914571
XCAW_02612	bglX	Beta-glucosidase-related glycosidase	-1.614311211
XCAW_00857	acoA	Thiamine pyrophosphate-dependent dehydrogenase, E1 component alpha subunit	-1.610824332
XCAW_01549	yapH	filamentous hemagglutinin-related protein	-1.606880959
XCAW_03382	XCAW_03382	Hypothetical Protein	-1.598262704
XCAW_00109	fabG	Dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases)	-1.588692921
XCAW_03407	cirA	Outer membrane receptor protein, mostly Fe transport	-1.58604605
XCAW_04454	XCAW_04454	Hypothetical Protein	-1.585602168

Table 3-10. Genes differentially expressed between strains *X. citri* subsp. *citri* str. 306 (A) and *X. citri* subsp. *citri* str. A^w 12879 (W) in NB (nutrient rich) medium. Cut off value of Log2 fold Change WNB/ANB ≥1 for overexpressed genes and ≤-1 for underexpressed genes. Log2 fold change 1 (| fold change | 2)

Locus tag	Gene Name	Product	Log_2 Fold Change WNB/ANB
Overexpressed Genes			
XAC2219	XAC2219	hypothetical protein	25.64823906
XAC4248	*gnl*	gluconolactonase	10.50680576
XAC1780	*amiC*	N-acetylmuramoyl-L-alanine amidase	7.433611332
XAC3260	*mobL*	plasmid mobilization protein	6.249044118
XAC2372	XAC2372	IS1479 transposase	4.188812122
XAC3474	*cit1*	citrate carrier protein	2.51470588
XAC3445	XAC3445	transcriptional regulator	2.499307593
XAC3475	XAC3475	hypothetical protein	2.485473632
XAC1165	XAC1165	hypothetical protein	2.383179166
XAC0338	XAC0338	hypothetical protein	2.276943861
XAC0398	*hrpD6*	HrpD6 protein	2.189413045
XAC1338	XAC1338	oxidoreductase	2.174773081
XAC0295	XAC0295	hypothetical protein	2.152862049
XAC1576	*pstC*	ABC transporter phosphate permease	2.126602949
XAC3489	*fyuA*	TonB-dependent receptor	2.11056609
XAC1575	*pstA*	ABC transporter phosphate permease	2.000651353
XAC1579	*oprO*	polyphosphate-selective porin O	1.99014292
XAC3712	XAC3712	metallopeptidase	1.987497004
XAC3757	XAC3757	hypothetical protein	1.938891939
XAC0999	*cirA*	colicin I receptor	1.927565575
XAC0310	*vanB*	vanillate O-demethylase	1.924455658
XAC2835	*mocA*	oxidoreductase	1.914657242
XAC0509	XAC0509	MFS transporter	1.911547087
XAC1792	*phoX*	alkaline phosphatase	1.834962219
XAC2520	XAC2520	TonB-dependent receptor	1.803981048
XAC0822	XAC0822	hypothetical protein	1.798432741
XAC1577	*pstS*	phosphate ABC transporter substrate-binding protein	1.774309607
XAC2538	XAC2538	hypothetical protein	1.753277012
XAC2113	XAC2113	hypothetical protein	1.749331387
XAC2860	XAC2860	hypothetical protein	1.739794559
XAC3444	*btuB*	TonB-dependent receptor	1.737743201
XAC2142	*lytS*	two-component system sensor protein	1.703861969
XAC1574	*pstB*	phosphate transporter ATP-binding protein	1.693102281
XAC0654	*acoR*	transcriptional regulator AcoR	1.615372055
XAC2763	XAC2763	extracellular protease	1.572651346
XAC3770	XAC3770	hypothetical protein	1.569563197

Table 3-10. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WNB/ANB
XAC1266	*hrpXct*	HrpX protein	1.568267693
XAC3501	XAC3501	hypothetical protein	1.566239878
XAC0162	*dctP*	C4-dicarboxylate transport system	1.544128974
XAC1164	XAC1164	hypothetical protein	1.515862882
XAC4355	XAC4355	hypothetical protein	1.510814239
XAC0298	XAC0298	hypothetical protein	1.500278696
XAC2604	XAC2604	ISxac4 transposase	1.496170371
XAC3028	XAC3028	histidine kinase-response regulator hybrid protein	1.495944706
XAC3612	XAC3612	peptidase	1.491243942
XAC2141	*lytT*	two-component system regulatory protein	1.487417813
XAC1104	*mobL*	plasmid mobilization protein	1.483732088
XAC3490	XAC3490	amylosucrase or alpha amylase	1.474480131
XAC3384	*pilN*	fimbrial assembly membrane protein	1.456476503
XAC1137	*prpB*	2-methylisocitrate lyase	1.455283697
XAC3000	*soxR*	SoxR family transcriptional regulator	1.436934908
XAC0300	XAC0300	serine-pyruvate aminotransferase	1.42513523
XAC3382	*pilP*	fimbrial assembly protein	1.422386632
XAC3754	XAC3754	hypothetical protein	1.419796784
XAC3610	*rhlE*	ATP-dependent RNA helicase	1.410457152
XAC3383	*pilO*	fimbrial assembly membrane protein	1.396824265
XAC2423	XAC2423	IS1478 transposase	1.389696936
XAC4009	*argI*	arginase	1.381846267
XAC0208	*ntrC*	two-component system regulatory protein	1.377969174
XAC3312	XAC3312	glycosyl hydrolase	1.367144662
XAC3473	XAC3473	sensor histidine kinase	1.366914397
XAC4127	*pknB*	serine/threonine kinase	1.358906214
XAC3704	XAC3704	DNA polymerase-like protein	1.353354957
XAC3647	*pheA*	chorismate mutase	1.348736132
XAC2313	XAC2313	LacI family transcriptional regulator	1.34456622
XAC1196	*lexA*	LexA repressor	1.336678942
XAC3772	XAC3772	LysR family transcriptional regulator	1.333027805
XAC1042	*phoB*	two-component system regulatory protein	1.328422784
XAC4257	*xylP*	transporter	1.325096356
XAC4369	*phoC*	phosphatase	1.321836272
XAC3035	XAC3035	hypothetical protein	1.320327487
XAC1910	*cirA*	TonB-dependent receptor	1.313206942
XAC3381	*pilQ*	fimbrial assembly protein	1.308210585
XAC2537	XAC2537	peptidase	1.306338218
XAC1130	*trpE*	hypothetical protein	1.299799316
XAC3958	XAC3958	hypothetical protein	1.29895361

Table 3-10. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WNB/ANB
XAC3640	*ybjZ*	ABC transporter ATP-binding protein	1.298024445
XAC1068	*stf*	phage-related tail protein	1.292866904
XAC3240	*fimA*	fimbrillin	1.28994735
XAC4150	*nodL*	nodulation protein	1.289643923
XAC0446	*pdhA*	pyruvate dehydrogenase E1 alpha subunit	1.288140846
XAC3428	XAC3428	hydrolase	1.28366189
XAC0508	XAC0508	LysR family transcriptional regulator	1.279977726
XAC0299	XAC0299	hypothetical protein	1.279487405
XAC3819	*gst*	glutathione S-transferase	1.261668538
XAC2500	XAC2500	LacI family transcriptional regulator	1.256706948
XAC0445	*pdhB*	pyruvate dehydrogenase E1 beta subunit	1.250592984
XAC3064	XAC3064	hypothetical protein	1.247208635
XAC1250	*obgE*	GTPase ObgE	1.244070086
XAC0970	*tuf*	elongation factor Tu	1.241899788
XAC2302	XAC2302	hypothetical protein	1.236512043
XAC0274	XAC0274	nuclease	1.235937581
XAC2773	*oar*	Oar protein	1.232672974
XAC2324	*cycW*	ABC transporter heme permease	1.228831091
XAC0168	*kduI*	5-keto-4-deoxyuronate isomerase	1.228597531
XAC1635	*hutU*	urocanate hydratase	1.227454133
XAC3686	XAC3686	hypothetical protein	1.226668753
XAC1199	*dnaE2*	DNA polymerase III subunit alpha	1.220359381
XAC3404	XAC3404	hypothetical protein	1.220003008
XAC3339	*cysB*	transcriptional regulator CysB-like protein	1.218857138
XAC3791	*yncA*	phosphinothricin acetyltransferase	1.218502295
XAC1637	*hutH*	histidine ammonia-lyase	1.218396454
XAC4229	*rspA*	starvation sensing protein	1.214754425
XAC2490	XAC2490	hypothetical protein	1.212956573
XAC3563	*rimI*	ribosomal-protein-alanine acetyltransferase	1.211869181
XAC4354	*yhdG*	amino acid transporter	1.208223002
XAC1774	XAC1774	hypothetical protein	1.206641764
XAC2536	XAC2536	hypothetical protein	1.206097283
XAC1776	*xylA*	xylose isomerase	1.204464335
XAC4272	XAC4272	LacI family transcriptional regulator	1.204316412
XAC2411	*acvB*	virulence protein	1.20359303
XAC2974	*ptsN*	nitrogen regulatory IIA protein	1.199204521
XAC4190	*fucP*	fucose permease	1.198482604
XAC1265	*hrpG*	HrpG protein	1.194586105
XAC0870	XAC0870	hypothetical protein	1.193665805
XAC2914	XAC2914	hypothetical protein	1.193244983
XAC2949	XAC2949	calcium-binding protein	1.192126089

Table 3-10. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WNB/ANB
XAC3331	*cysI*	sulfite reductase subunit beta	1.191296014
XAC3860	XAC3860	N-acetylmuramoyl-L-alanine amidase	1.188397814
XAC2312	XAC2312	hypothetical protein	1.185492429
XAC2848	XAC2848	hypothetical protein	1.185372734
XAC3166	*bfeA*	ferric enterobactin receptor	1.184298653
XAC1286	XAC1286	alpha-L-arabinofuranosidase	1.18405598
XAC3928	XAC3928	hypothetical protein	1.182582034
XAC0169	*kduD*	short chain dehydrogenase	1.17965288
XAC1240	XAC1240	hypothetical protein	1.177239607
XAC3433	XAC3433	hypothetical protein	1.177010501
XAC4168	*hetI*	HetI protein	1.176989353
XAC3316	XAC3316	tRNA/rRNA methyltransferase	1.175175543
XAC1232	XAC1232	DNA-3-methyladenine glycosylase	1.174198144
XAC3995	*acrE*	acriflavin resistance protein	1.174020371
XAC1512	XAC1512	serine peptidase	1.173302695
XAC2826	XAC2826	alcohol dehydrogenase	1.171771584
XAC2589	*pheT*	phenylalanyl-tRNA synthetase subunit beta	1.1717389
XAC2341	*gaa*	glutaryl-7-ACA acylase	1.17093649
XAC3733	XAC3733	NtrC family transcriptional regulator	1.167496149
XAC3863	XAC3863	hypothetical protein	1.166631626
XAC1616	XAC1616	hypothetical protein	1.165436305
XAC1449	XAC1449	hypothetical protein	1.165224046
XAC0871	XAC0871	hypothetical protein	1.165180569
XAC1636	*hutG*	formylglutamate amidohydrolase	1.164738375
XAC1793	*celD*	glucan 1,4-beta-glucosidase	1.164303373
XAC1329	*rumA*	23S rRNA 5-methyluridine methyltransferase	1.163782594
XAC3721	XAC3721	D-amino acid oxidase	1.163509009
XAC3391	*recG*	ATP-dependent DNA helicase RecG	1.161813634
XAC3072	*fucA1*	alpha-L-fucosidase	1.159787168
XAC1794	*sglT*	sodium/glucose cotransport protein	1.157757657
XAC1129	*fabF*	3-oxoacyl-ACP synthase	1.157564842
XAC1851	*mvaB*	hydroxymethylglutaryl-CoA lyase	1.157385744
XAC3909	*dpm1*	dolichol-phosphate mannosyltransferase	1.155608746
XAC1640	*hutC*	histidine utilization repressor	1.155113338
XAC0025	XAC0025	hypothetical protein	1.151633231
XAC3505	*rhgB*	rhamnogalacturonase B	1.151359093
XAC3385	*pilM*	fimbrial assembly membrane protein	1.150285388
XAC1638	*hutI*	imidazolonepropionase	1.149561775
XAC0723	*dsbA*	disulfide oxidoreductase	1.149165434
XAC3923	*speA*	arginine decarboxylase	1.149105259
XAC0554	XAC0554	hypothetical protein	1.14870911

Table 3-10. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WNB/ANB
XAC3673	XAC3673	histidine kinase	1.148084876
XAC1835	*hisI*	bifunctional phosphoribosyl-AMP cyclohydrolase/phosphoribosyl-ATP pyrophosphatase	1.147122502
XAC3476	*ybhD*	transcriptional regulator	1.145371713
XAC1471	XAC1471	hypothetical protein	1.143158199
XAC0202	XAC0202	hypothetical protein	1.142130292
XAC4276	*pyrF*	orotidine 5'-phosphate decarboxylase	1.141059115
XAC4199	XAC4199	polyvinylalcohol dehydrogenase	1.141037777
XAC0017	XAC0017	hypothetical protein	1.14056938
XAC3313	*susB*	alpha-glucosidase	1.140338807
XAC1777	*xylE*	MFS transporter	1.139337986
XAC2752	*yxaH*	transporter	1.138867614
XAC3125	XAC3125	hypothetical protein	1.138848561
XAC2531	*btuB*	TonB-dependent receptor	1.138552214
XAC1362	*nerA*	GTN reductase	1.138250538
XAC3862	*tcbD*	chloromuconate cycloisomerase	1.136972174
XAC2707	*tpiA*	triosephosphate isomerase	1.135555794
XAC2830	*fhuA*	TonB-dependent receptor	1.135551606
XAC3341	*cysK*	cysteine synthase	1.135197438
XAC4300	XAC4300	hypothetical protein	1.135139198
XAC3578	*ipsI*	IpsJ protein	1.134798188
XAC0070	XAC0070	ankyrin-like protein	1.134434828
XAC3241	*fimA*	fimbrillin	1.133014942
XAC2303	XAC2303	beta-alanine synthetase	1.132882701
XAC1798	*regS*	two-component system sensor protein	1.132438074
XAC0251	XAC0251	TetR family transcriptional regulator	1.132354602
XAC0022	*serA*	D-3-phosphoglycerate dehydrogenase	1.131665742
XAC0600	*cycA*	D-alanine/D-serine/glycine permease	1.131332249
XAC2824	XAC2824	phosphodiesterase-nucleotide pyrophosphatase	1.131121447
XAC4302	*folE*	GTP cyclohydrolase I	1.131109687
XAC2803	*baeR*	two-component system regulatory protein	1.130857165
XAC1113	*slp*	outer membrane protein Slp	1.130749707
XAC3851	XAC3851	hypothetical protein	1.130178883
XAC2194	XAC2194	hypothetical protein	1.130073359
XAC3376	XAC3376	hypothetical protein	1.130040329
XAC1838	XAC1838	enolase	1.129924845
XAC3379	*moxR*	methanol dehydrogenase regulatory protein	1.128594684
XAC2530	XAC2530	hypothetical protein	1.12768757

Table 3-10. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WNB/ANB
XAC4187	XAC4187	2-hydroxyhepta-2,4-diene-1, 7-dioate isomerase	1.127324344
XAC2522	*egl2*	cellulase	1.12713615
XAC0952	*pth*	peptidyl-tRNA hydrolase	1.127058666
XAC2877	XAC2877	pirin	1.126419463
XAC3625	*fabB*	beta-ketoacyl-[ACP] synthase I	1.126179244
XAC3083	XAC3083	hypothetical protein	1.125500518
XAC0254	*yjl094C*	Na+/H+-exchanging protein	1.125157903
XAC1752	XAC1752	hypothetical protein	1.125079028
XAC3429	*argD*	acetylornithine transaminase	1.125035412
XAC2019	XAC2019	hypothetical protein	1.12440991
XAC2783	*trx*	thioredoxin	1.123710113
XAC0869	XAC0869	acetoin utilization family protein	1.12367105
XAC3007	XAC3007	hypothetical protein	1.123561922
XAC1582	*nth*	endonuclease III	1.123312544
XAC3850	*acrA*	acriflavin resistance protein	1.121889686
XAC2744	XAC2744	phytoene dehydrogenase	1.121436285
XAC2018	XAC2018	hypothetical protein	1.121230828
XAC3577	*ipsJ*	IpsJ protein	1.121047213
XAC3194	*btuB*	vitamin B transport outer membrane protein	1.1208382
XAC3481	*afuA*	periplasmic iron-binding protein	1.120663184
XAC2391	*apt*	adenine phosphoribosyltransferase	1.119220893
XAC0544	XAC0544	hypothetical protein	1.119092736
XAC2329	*dsbE*	C-type cytochrome biogenesis protein/thioredoxin	1.118456085
XAC2720	*truA*	tRNA pseudouridine synthase A	1.118171756
XAC3421	*acoK*	transcriptional regulator	1.117750374
XAC1022	*tdh*	L-threonine 3-dehydrogenase	1.117170335
XAC3465	*htrB*	lipid A biosynthesis lauroyl acyltransferase	1.116705322
XAC3151	XAC3151	hypothetical protein	1.11644408
XAC1204	XAC1204	alanyl dipeptidyl peptidase	1.11623036
XAC2455	*petC*	ubiquinol cytochrome C oxidoreductase cytochrome C1 subunit	1.115582552
XAC2676	XAC2676	hypothetical protein	1.115566883
XAC2809	XAC2809	hypothetical protein	1.115184493
XAC3327	XAC3327	RND efflux membrane fusion protein	1.114652792
XAC0348	XAC0348	transferase	1.114438071
XAC1619	XAC1619	hypothetical protein	1.113421941
XAC1315	XAC1315	enoyl-CoA hydratase	1.112931913
XAC3705	XAC3705	hypothetical protein	1.112584024

Table 3-10. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WNB/ANB
		ubiquinol cytochrome C oxidoreductase	
XAC2456	*petB*	cytochrome B subunit	1.112583372
XAC1843	XAC1843	hypothetical protein	1.112096495
XAC0450	XAC0450	hypothetical protein	1.111816831
XAC2973	XAC2973	sigma-54 modulation protein	1.11146047
XAC1252	*mviN*	virulence factor	1.111353917
XAC0174	*phhA*	phenylalanine 4-monooxygenase	1.111002556
XAC4230	*xylB*	arabinosidase	1.110191
XAC2976	XAC2976	hypothetical protein	1.109765725
XAC3913	*dut*	deoxyuridine 5'-triphosphate nucleotidohydrolase	1.109605606
XAC0969	*fusA*	elongation factor G	1.109284519
XAC0615	XAC0615	aminopeptidase	1.109124905
XAC0454	*hmgA*	homogentisate 1,2-dioxygenase	1.10906224
XAC0452	XAC0452	4-hydroxyphenylpyruvate dioxygenase	1.108994714
XAC3644	*glmU*	UDP-N-acetylglucosamine pyrophosphorylase	1.108805102
XAC3709	*wrbA*	tryptophan repressor-binding protein	1.107551541
XAC3987	XAC3987	leucine aminopeptidase	1.10686642
XAC4373	*rnpA*	ribonuclease P	1.106071395
XAC3463	*tolC*	TolC protein	1.105906894
XAC1239	*ate1*	arginyl-tRNA-protein transferase	1.105584562
XAC2305	*traB*	pheromone shutdown protein	1.104288688
XAC3510	*def*	peptide deformylase	1.103200718
XAC2717	*trpB*	tryptophan synthase subunit beta	1.102727484
XAC3887	*ctaD*	cytochrome C oxidase subunit I	1.102617787
XAC2532	XAC2532	peptidase	1.102241954
XAC2681	*nadC*	nicotinate-nucleotide pyrophosphorylase	1.101622788
XAC0813	*metK*	S-adenosylmethionine synthetase	1.101588425
XAC1797	*regR*	two-component system regulatory protein	1.101549049
XAC3869	*bglX*	beta-glucosidase	1.101506779
XAC2016	XAC2016	hypothetical protein	1.101444505
XAC2727	XAC2727	hypothetical protein	1.101254486
XAC2545	*pepQ*	proline dipeptidase	1.100225884
XAC3335	XAC3335	sensor histidine kinase	1.100053613
XAC2590	*pheS*	phenylalanyl-tRNA synthetase subunit alpha	1.099938956
XAC0731	XAC0731	hypothetical protein	1.099891801
XAC3347	*pgk*	phosphoglycerate kinase	1.099637504
XAC0247	XAC0247	acyltransferase	1.09896329
XAC2496	XAC2496	hypothetical protein	1.097121352
XAC3813	*sppA*	endopeptidase IV	1.096928553

Table 3-10. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WNB/ANB
XAC2398	XAC2398	hypothetical protein	1.095908599
XAC1314	*paaF*	enoyl-CoA hydratase	1.095898799
XAC2015	*ndk*	nucleoside diphosphate kinase	1.095855212
XAC3189	*cobC*	threonine-phosphate decarboxylase	1.095574873
XAC1713	XAC1713	carboxypeptidase-like protein	1.095421852
XAC3455	*leuA*	2-isopropylmalate synthase	1.094712104
XAC3487	*cebR*	transcriptional regulator	1.093784343
XAC2908	*murD*	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	1.092905914
XAC0129	*aldA*	chloroacetaldehyde dehydrogenase	1.092346947
XAC2999	XAC2999	peptidase	1.092100333
XAC3069	XAC3069	16S ribosomal RNA methyltransferase RsmE	1.092020946
XAC3073	XAC3073	hypothetical protein	1.091729241
XAC0479	XAC0479	hypothetical protein	1.091305768
XAC1032	*purF*	amidophosphoribosyltransferase	1.091145302
XAC2806	XAC2806	beta-lactamase	1.090264921
XAC2965	*murA*	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	1.090095408
XAC3836	XAC3836	hypothetical protein	1.090065475
XAC1716	*pyrG*	CTP synthetase	1.089830993
XAC2735	*rimO*	ribosomal protein S12 methylthiotransferase	1.089493733
XAC2020	XAC2020	hypothetical protein	1.089033283
XAC2345	*argH*	argininosuccinate lyase	1.089027511
XAC0233	*fabH*	3-oxoacyl-ACP synthase	1.08888636
XAC1556	*fucP*	glucose-galactose transporter	1.088570635
XAC0591	XAC0591	dipeptidyl peptidase IV	1.088545568
XAC2540	XAC2540	hypothetical protein	1.087660409
XAC2931	XAC2931	hypothetical protein	1.087452638
XAC3438	*pfkA*	6-phosphofructokinase	1.087005223
XAC3239	*pilB*	pilus biogenesis protein	1.086446644
XAC3326	*acrF*	acriflavin resistance protein	1.086060973
XAC2959	*purM*	phosphoribosylaminoimidazole synthetase	1.085947286
XAC4299	XAC4299	hypothetical protein	1.085694007
XAC1090	*dnaQ*	DNA polymerase III subunit epsilon	1.085589405
XAC1139	*acnA*	aconitate hydratase	1.085443877
XAC0751	*nusB*	transcription antitermination protein NusB	1.085066611
XAC3302	*thiG*	thiazole synthase	1.084474739
XAC0005	XAC0005	hypothetical protein	1.084119954
XAC3848	*mtrC*	membrane fusion protein	1.083463936
XAC0024	XAC0024	hypothetical protein	1.083100501

Table 3-10. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WNB/ANB
XAC2745	XAC2745	metallopeptidase	1.082914289
XAC3375	XAC3375	hypothetical protein	1.082173323
XAC2730	XAC2730	hypothetical protein	1.081848637
XAC0635	XAC0635	hypothetical protein	1.081745394
XAC2678	*purK*	phosphoribosylaminoimidazole carboxylase ATPase subunit	1.08168274
XAC1043	XAC1043	hypothetical protein	1.081681589
XAC4022	*phoQ*	two-component system sensor protein	1.081456768
XAC3849	*acrD*	acriflavin resistance protein	1.081189028
XAC4370	*trmE*	tRNA modification GTPase TrmE	1.080532425
XAC3352	*gapA*	glyceraldehyde-3-phosphate dehydrogenase	1.080132309
XAC3871	*gcp*	DNA-binding/iron metalloprotein/AP endonuclease	1.079686537
XAC2691	*nuoN*	NADH dehydrogenase subunit N	1.078286647
XAC2713	XAC2713	oxidoreductase	1.077798924
XAC1262	XAC1262	hypothetical protein	1.077651629
XAC2881	*cstA*	carbon starvation protein A	1.077483608
XAC0179	*ylmA*	ABC transporter ATP-binding protein	1.077426751
XAC1201	XAC1201	hypothetical protein	1.077311182
XAC3075	XAC3075	beta-mannosidase	1.077302533
XAC1282	XAC1282	two-component system sensor protein	1.076618737
XAC2022	*moeA*	molybdopterin biosynthesis	1.075617702
XAC2461	XAC2461	(dimethylallyl)adenosine tRNA methylthiotransferase	1.075603939
XAC1214	*gcvP*	glycine dehydrogenase	1.074795455
XAC0943	XAC0943	hypothetical protein	1.074478864
XAC2542	*yveA*	amino acid permease	1.074168054
XAC2905	*ssb*	single-stranded DNA-binding protein	1.073988941
XAC2703	*nuoB*	NADH dehydrogenase subunit B	1.073581745
XAC1004	*typA*	GTP-binding elongation factor protein	1.072728238
XAC2846	XAC2846	FUR family transcriptional regulator	1.072522739
XAC0944	*prfA*	peptide chain release factor 1	1.072466415
XAC2594	*thrS*	threonyl-tRNA synthetase	1.072117274
XAC3560	*btuB*	TonB-dependent receptor	1.07058876
XAC2980	*mgtE*	Mg++ transporter	1.070304477
XAC3047	*mtgA*	monofunctional biosynthetic peptidoglycan transglycosylase	1.069932542
XAC2106	XAC2106	hypothetical protein	1.069628899
XAC0668	*lipA*	lipoyl synthase	1.069207751
XAC4004	XAC4004	peptidase	1.069193407

Table 3-10. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WNB/ANB
XAC3637	*glmS*	glucosamine--fructose-6-phosphate aminotransferase	1.068626998
XAC1409	*lpxA*	UDP-N-acetylglucosamine acyltransferase	1.068600973
XAC2008	*lolA*	outer-membrane lipoprotein carrier protein	1.068320953
XAC2543	XAC2543	hypothetical protein	1.067962745
XAC2909	XAC2909	hypothetical protein	1.067469176
XAC0743	*glyA*	serine hydroxymethyltransferase	1.067307853
XAC3442	*ppa*	inorganic pyrophosphatase	1.067266351
XAC0628	XAC0628	prolyl oligopeptidase	1.066947622
XAC0863	*ksgA*	dimethyladenosine transferase	1.066817843
XAC4023	*phoP*	two-component system regulatory protein	1.066440427
XAC1109	*dnaX*	DNA polymerase III subunits gamma and tau	1.06612866
XAC3671	XAC3671	nucleotide-binding protein	1.065581085
XAC2878	XAC2878	hypothetical protein	1.065339592
XAC3145	*tolQ*	TolQ protein	1.065185305
XAC3118	XAC3118	hypothetical protein	1.064501937
XAC2847	*gltX*	glutamyl-tRNA synthetase	1.064433445
XAC3607	*uptC*	type II secretion system protein-like protein	1.064325332
XAC3526	*gtrB*	glycosyl transferase	1.064008591
XAC0788	*secA*	preprotein translocase subunit SecA	1.063402126
XAC2053	*tex*	transcription-like protein	1.063354974
XAC1826	*hisS*	histidyl-tRNA synthetase	1.063333921
XAC2544	XAC2544	hypothetical protein	1.063098061
XAC3071	*iroN*	TonB-dependent receptor	1.063003868
XAC0685	XAC0685	histidine kinase-response regulator hybrid protein	1.062866613
XAC1017	*sbp*	sulfate ABC transporter substrate-binding protein	1.061872022
XAC3357	XAC3357	beta-lactamase	1.061762926
XAC1428	*map*	methionine aminopeptidase	1.061644888
XAC4217	*tatB*	sec-independent translocase	1.061559573
XAC3835	*icd*	isocitrate dehydrogenase	1.06143259
XAC1430	*dapD*	2,3,4,5-tetrahydropyridine-2,6-carboxylate N-succinyltransferase	1.060649377
XAC2001	*clpA*	ATP-dependent Clp protease subunit	1.060164854
XAC1992	XAC1992	c-di-GMP phosphodiesterase A	1.05948882
XAC3992	XAC3992	hypothetical protein	1.05937852
XAC3602	*metB*	cystathionine gamma-synthase	1.059208377
XAC3603	*cysB*	cystathionine beta-synthase	1.0583837
XAC1000	*dhs1*	family II 2-keto-3-deoxy-D-arabino-heptulosonate 7-phosphate synthase	1.058345599

Table 3-10. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WNB/ANB
XAC1539	*purB*	adenylosuccinate lyase	1.055413001
XAC1238	XAC1238	hypothetical protein	1.055020012
XAC3110	XAC3110	glycosyltransferase	1.053902078
XAC0382	*aspH*	aspartyl-asparaginyl beta-hydroxylase	1.053845461
XAC3494	*aspC*	aminotransferase	1.05375416
XAC3141	*ompP6*	outer membrane protein P6	1.053574987
XAC2692	*nuoM*	NADH dehydrogenase subunit M	1.053403547
XAC2683	*pnp*	polynucleotide phosphorylase	1.053215605
XAC1732	*hflB*	cell division protein	1.0531697
XAC2687	*infB*	translation initiation factor IF-2	1.052806459
XAC1804	*murB*	UDP-N-acetylenolpyruvoylglucosamine reductase	1.052697606
XAC0221	*secB*	preprotein translocase subunit SecB	1.052151509
XAC0645	*pepN*	aminopeptidase	1.051919733
XAC2698	*nuoG*	NADH dehydrogenase subunit G	1.05135027
XAC1880	*rpfB*	long-chain fatty acid-CoA ligase	1.050951518
XAC2360	*pyrC*	dihydroorotase	1.050475376
XAC2758	*gltT*	glutamate symporter	1.050231252
XAC1627	*ligA*	NAD-dependent DNA ligase LigA	1.04985178
XAC1155	*hflK*	integral membrane protease subunit	1.04957679
XAC3801	*def*	peptide deformylase	1.049531524
XAC2701	*nuoD*	NADH dehydrogenase subunit D	1.049092169
XAC1040	*ppk*	polyphosphate kinase	1.048546324
XAC2700	*nuoE*	NADH dehydrogenase subunit E	1.047166009
XAC1590	XAC1590	hypothetical protein	1.046648045
XAC4006	*trpS*	tryptophanyl-tRNA synthetase	1.046271613
XAC2924	*pilT*	twitching motility protein	1.04616725
XAC3493	XAC3493	ribosome-associated GTPase	1.045114872
XAC4367	*glpQ*	glycerophosphoryl diester phosphodiesterase	1.041950638
XAC1740	*recA*	recombinase A	1.041511744
XAC2805	*yjl094C*	cation:proton antiporter	1.041157483
XAC3393	*spoT*	pentaphosphate guanosine-3'-pyrophosphohydrolase	1.040972721
XAC3912	*algC*	phosphomannomutase	1.03961513
XAC0950	*prsA*	ribose-phosphate pyrophosphokinase	1.039604702
XAC2971	*yhbG*	ABC transporter ATP-binding protein	1.039398199
XAC3897	*tyrS*	tyrosyl-tRNA synthetase	1.039046534
XAC2743	*oar*	Oar protein	1.036089137
XAC3650	*atpG*	ATP synthase F0F1 subunit gamma	1.035872908
XAC0120	*tldD*	TldD protein	1.033932809

Table 3-10. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WNB/ANB
XAC0989	*rpsE*	30S ribosomal protein S5	1.033905073
XAC0893	*glnS*	glutaminyl-tRNA synthetase	1.02946677
XAC4284	*mdoD*	glucan biosynthesis protein D	1.027224464
XAC0966	*rpoC*	DNA-directed RNA polymerase subunit beta'	1.024602274
XAC1551	*ugd*	UDP-glucose dehydrogenase	1.023935428
XAC0965	*rpoB*	DNA-directed RNA polymerase subunit beta	1.019572533
XAC1623	*smc*	chromosome segregation protein	1.019266918
XAC2298	*rpsA*	30S ribosomal protein S1	1.014668444
Underexpressed Genes			
XAC1492	XAC1492	hypothetical protein	-10.21684841
XAC3594	XAC3594	hypothetical protein	-7.376270824
XAC3593	XAC3593	NAD dependent epimerase/dehydratase/dehydrogenase	-6.332084605
XAC1507	*mobL*	plasmid mobilization protein	-3.281412651
XAC2868	*vieA*	response regulator	-3.275599917
XAC0209	*yojM*	superoxide dismutase like protein	-3.275599917
XAC0048	XAC0048	hypothetical protein	-3.031674789
XAC2866	*mcp*	chemotaxis protein	-3.016953925
XAC0334	*sflA*	NADH-dependent FMN reductase	-2.746943112
XAC3273	XAC3273	histidine kinase-response regulator hybrid protein	-2.657889437
XAC2865	*cheA*	chemotaxis histidine protein kinase	-2.655987946
XAC1891	*tsr*	chemotaxis protein	-2.260813095
XAC3323	XAC3323	hypothetical protein	-2.235229866
XAC2657	XAC2657	hypothetical protein	-2.158660687
XAC0335	XAC0335	hypothetical protein	-2.151642626
XAC0029	*egl*	cellulase	-2.139857262
XAC0028	*egl*	cellulase	-2.12773726
XAC1900	*tsr*	chemotaxis protein	-2.07995337
XAC3784	XAC3784	hypothetical protein	-1.97520681
XAC1899	*tsr*	chemotaxis protein	-1.964518333
XAC1896	*tsr*	chemotaxis protein	-1.959713612
XAC0210	*sodC2*	superoxide dismutase	-1.915635997
XAC1508	XAC1508	hypothetical protein	-1.913913416
XAC0691	XAC0691	hypothetical protein	-1.904667668
XAC0092	XAC0092	hypothetical protein	-1.883234536
XAC2599	*aglA*	alpha-glucosidase	-1.867743642
XAC0098	XAC0098	hypothetical protein	-1.843399276
XAC0756	*kdpA*	potassium-transporting ATPase subunit A	-1.835669295
XAC1890	*cheR*	chemotaxis protein methyltransferase	-1.831555873
XAC0050	XAC0050	hypothetical protein	-1.781027304

Table 3-10. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WNB/ANB
XAC3693	*motA*	flagellar motor protein MotA	-1.778136017
XAC1146	*fecA*	TonB-dependent receptor	-1.741489966
XAC1795	XAC1795	hypothetical protein	-1.719416095
XAC0336	*metE*	5-methyltetrahydropteroyltriglutamate--homocysteine S-methyltransferase	-1.709709626
XAC2448	*mcp*	chemotaxis protein	-1.709265955
XAC3741	XAC3741	hypothetical protein	-1.703514133
XAC0350	XAC0350	hypothetical protein	-1.700503313
XAC2600	*btuB*	TonB-dependent receptor	-1.697158215
XAC1815	*fhaB*	filamentous hemagglutinin	-1.679244522
XAC1887	*pdeA*	c-di-GMP phosphodiesterase A	-1.672056227
XAC3365	XAC3365	hypothetical protein	-1.671525209
XAC1895	*tsr*	chemotaxis protein	-1.653756018
XAC3635	XAC3635	hypothetical protein	-1.651255644
XAC2869	*cheR*	response regulator for chemotaxis	-1.642201143
XAC0611	*tsr*	chemotaxis protein	-1.627313159
XAC2597	*suc1*	transporter	-1.613187569
XAC2482	*rrpX*	transcriptional regulator	-1.605625375
XAC1902	*tsr*	chemotaxis protein	-1.592615948
XAC3725	XAC3725	hypothetical protein	-1.584295514
XAC1894	*tsr*	chemotaxis protein	-1.570873059
XAC0051	*asnB*	asparagine synthase	-1.569680723
XAC0047	XAC0047	galactosyltransferase	-1.559099776
XAC0424	XAC0424	hypothetical protein	-1.552388457
XAC3726	XAC3726	hypothetical protein	-1.519064447
XAC1993	XAC1993	hypothetical protein	-1.514798533
XAC1889	*cheD*	chemoreceptor glutamine deamidase CheD	-1.503470372
XAC3922	*entF*	ATP-dependent serine activating enzyme	-1.496272831
XAC0606	XAC0606	endonuclease	-1.493931709
XAC0496	XAC0496	hypothetical protein	-1.487092175
XAC0235	*dhaA*	haloalkane dehalogenase	-1.483694734
XAC3132	*mcp*	chemotaxis protein	-1.474166582
XAC3364	XAC3364	hypothetical protein	-1.470066015
XAC3927	XAC3927	hypothetical protein	-1.469846529
XAC2598	XAC2598	hypothetical protein	-1.468776033
XAC2027	XAC2027	hypothetical protein	-1.468136632
XAC1509	XAC1509	hypothetical protein	-1.454251212
XAC1971	XAC1971	hypothetical protein	-1.453872449
XAC4283	XAC4283	sensor histidine kinase	-1.453084866
XAC2602	*aglA*	alpha-glucosidase	-1.433527863
XAC1903	*cheA*	chemotaxis protein	-1.389816094

Table 3-10. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WNB/ANB
XAC0940	XAC0940	hypothetical protein	-1.389037155
XAC0108	*atsE*	AtsE protein	-1.382241601
XAC0957	*tuf*	elongation factor Tu	-1.376878728
XAC0540	XAC0540	ribonuclease	-1.372944605
XAC1193	XAC1193	hypothetical protein	-1.364648087
XAC0107	XAC0107	hypothetical protein	-1.360547243
XAC3562	*pel*	pectate lyase	-1.353706678
XAC3300	*estA*	esterase	-1.351414413
XAC2596	*cgt*	cyclomaltodextrin glucanotransferase	-1.344697177
XAC1034	XAC1034	peptidyl-Asp metalloendopeptidase	-1.33483264
XAC1893	*tsr*	chemotaxis protein	-1.329618489
XAC1178	XAC1178	oxidoreductase	-1.324489879
XAC3763	XAC3763	hypothetical protein	-1.317078785
XAC0690	*fecA*	TonB-dependent receptor	-1.316467244
XAC3272	XAC3272	hypothetical protein	-1.311918746
XAC2026	XAC2026	hypothetical protein	-1.311415153
XAC0285	XAC0285	hypothetical protein	-1.306554629
XAC2580	*gumG*	GumG protein	-1.30328786
XAC1996	*mcp*	chemotaxis protein	-1.300988651
XAC3050	*btuB*	TonB-dependent receptor	-1.298541812
XAC1905	XAC1905	hypothetical protein	-1.295249076
XAC1363	*araJ*	MFS transporter	-1.294341225
XAC2577	*gumJ*	GumJ protein	-1.287085498
XAC1904	*cheY*	chemotaxis response regulator	-1.28458781
XAC1973	*fliS*	flagellar protein	-1.281739723
XAC2582	*gumE*	GumE protein	-1.278122361
XAC0394	*hrpF*	HrpF protein	-1.27398998
XAC0584	XAC0584	hypothetical protein	-1.264705549
XAC1972	XAC1972	hypothetical protein	-1.263599233
XAC3739	XAC3739	hypothetical protein	-1.263549906
XAC0798	*amy*	alpha-amylase	-1.260223514
XAC3324	XAC3324	hypothetical protein	-1.255678443
XAC1364	XAC1364	hypothetical protein	-1.252810141
XAC1868	XAC1868	hypothetical protein	-1.251258578
XAC2494	*yieO*	drug resistance translocase	-1.25064029
XAC2585	*gumB*	GumB protein	-1.250118598
XAC0747	XAC0747	hypothetical protein	-1.246902249
XAC2584	*gumC*	GumC protein	-1.24510651
XAC0682	XAC0682	hypothetical protein	-1.243210188
XAC1177	XAC1177	hypothetical protein	-1.242395313
XAC2608	XAC2608	VirB6 protein	-1.233797995

Table 3-10. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WNB/ANB
XAC1666	*tsr*	chemotaxis protein	-1.23355901
XAC3615	XAC3615	hypothetical protein	-1.232611071
XAC3839	XAC3839	hypothetical protein	-1.227809416
XAC3446	XAC3446	hypothetical protein	-1.227739588
XAC1810	XAC1810	hypothetical protein	-1.226825166
XAC2261	*yme*	plasmid-like protein	-1.223865173
XAC2812	XAC2812	hypothetical protein	-1.222394917
XAC3966	XAC3966	hypothetical protein	-1.22211826
XAC2579	*gumH*	GumH protein	-1.219095235
XAC0096	XAC0096	hypothetical protein	-1.217779136
XAC3866	XAC3866	hypothetical protein	-1.214029647
XAC2359	XAC2359	hypothetical protein	-1.2101597
XAC0428	*malQ*	4-alpha-glucanotransferase	-1.206994407
XAC3254	*glgX*	glycogen debranching protein	-1.204536333
XAC1319	*algU*	RNA polymerase sigma factor RpoE	-1.198589677
XAC0612	*engXCA*	cellulase	-1.19584223
XAC1632	XAC1632	hypothetical protein	-1.193661007
XAC2581	*gumF*	GumF protein	-1.191547922
XAC2583	*gumD*	GumD protein	-1.18885598
XAC0610	XAC0610	histidine kinase-response regulator hybrid protein	-1.187522049
XAC2414	*lig3*	ATP-dependent DNA ligase	-1.18464461
XAC2103	XAC2103	DNA recombinase	-1.170631164
XAC1321	*mucD*	periplasmic protease	-1.165075851
XAC2574	*gumM*	GumM protein	-1.163844975
XAC4294	XAC4294	hypothetical protein	-1.162498052
XAC1521	*grpE*	heat shock protein GrpE	-1.161281031
XAC1654	*acpD*	ACP phosphodieterase	-1.160481153
XAC0465	XAC0465	metalloproteinase	-1.159209175
XAC2576	*gumK*	GumK protein	-1.156219719
XAC2578	*gumI*	GumI protein	-1.156150737
XAC3121	*fepA*	TonB-dependent receptor	-1.156043879
XAC2122	XAC2122	dehydrogenase	-1.143167003
XAC0035	XAC0035	hypothetical protein	-1.142783524
XAC0189	*iorA*	indolepyruvate ferredoxin oxidoreductase	-1.139472125
XAC0224	*poxB*	pyruvate dehydrogenase	-1.135612256
XAC1187	XAC1187	hydroxylase large subunit	-1.131635635
XAC0431	*glgX*	glycogen debranching protein	-1.129093782
XAC1211	*katE*	catalase	-1.128509742
XAC0155	XAC0155	trehalose synthase	-1.12365149
XAC3458	*leuC*	isopropylmalate isomerase large subunit	-1.122307106

Table 3-10. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WNB/ANB
XAC2893	*yagR*	oxidoreductase	-1.119474038
XAC0495	XAC0495	two-component system regulatory protein	-1.118411592
XAC2151	*yapH*	YapH protein	-1.113301035
XAC1188	XAC1188	hydroxylase molybdopterin-containing subunit	-1.11308543
XAC3524	XAC3524	hypothetical protein	-1.112176282
XAC1495	*xrvA*	virulence regulator	-1.102865964
XAC2992	XAC2992	endoproteinase ArgC	-1.102233164
XAC1959	XAC1959	hypothetical protein	-1.102019808
XAC0122	*tldD*	TldD protein	-1.101179671
XAC0154	XAC0154	alpha-amylase	-1.098686034
XAC2895	*yagT*	xanthine dehydrogenase iron-sulfur-binding subunit	-1.088586864
XAC4204	XAC4204	hypothetical protein	-1.083279682
XAC0223	XAC0223	hypothetical protein	-1.080628281
XAC1400	XAC1400	PHB depolymerase	-1.080212735
XAC0585	XAC0585	hypothetical protein	-1.078528777
XAC3243	*pilD*	type IV pre-pilin leader peptidase	-1.077050741
XAC0741	*yjjK*	ABC transporter ATP-binding protein	-1.072529493
XAC2990	XAC2990	hypothetical protein	-1.070915021
XAC1149	XAC1149	bacterioferritin	-1.06926196
XAC0264	*accD*	acyl-CoA carboxyltransferase subunit beta	-1.061441881
XAC2040	*yggB*	small conductance mechanosensitive ion channel	-1.061111518
XAC2951	*comEA*	DNA transport competence protein	-1.059680762
XAC4274	XAC4274	OmpA-like protein	-1.058356391
XAC1325	*rnc*	ribonuclease III	-1.042004593
XAC0711	XAC0711	GntR family transcriptional regulator	-1.034955794
XAC1012	*mopB*	hypothetical protein	-1.005153155

Table 3-11. Genes differentially expressed between strains *X. citri* subsp. *citri* str. 306 (A) and *X. citri* subsp. *citri* str. A^w 12879 (W) in XVM2 (hrp inducing) medium. Cut off value of Log2 fold Change WXVM/AXVM ≥ 1 for overexpressed genes and ≤ -1 for underexpressed genes. Log2 fold change 1 (| fold change| 2)

Locus tag	Gene Name	Product	Log_2 Fold Change WXVM/AXVM
Overexpressed Genes			
XAC4248	*gnl*	gluconolactonase	3.877771021
XAC1689	XAC1689	hypothetical protein	3.775079328
XAC3616	*bioD*	dithiobiotin synthetase	2.768199661
XAC3444	*btuB*	TonB-dependent receptor	2.626959105
XAC1696	XAC1696	methyltransferase	2.196872429
XAC2538	XAC2538	hypothetical protein	2.093287334
XAC2860	XAC2860	hypothetical protein	2.038647836
XAC2603	XAC2603	ISxac4 transposase	1.969683352
XAC2787	XAC2787	hypothetical protein	1.956849632
XAC2604	XAC2604	ISxac4 transposase	1.955367064
XAC1688	XAC1688	hypothetical protein	1.880628405
XAC3475	XAC3475	hypothetical protein	1.828477254
XAC3712	XAC3712	metallopeptidase	1.815121614
XAC2520	XAC2520	TonB-dependent receptor	1.798880957
XAC3445	XAC3445	transcriptional regulator	1.79169953
XAC2891	XAC2891	hypothetical protein	1.790255834
XAC0999	*cirA*	colicin I receptor	1.7665622
XAC3518	*bcsA*	celullose synthase	1.757482347
XAC3474	*cit1*	citrate carrier protein	1.73848736
XAC3028	XAC3028	histidine kinase-response regulator hybrid protein	1.594045007
XAC3090	XAC3090	leucin rich protein	1.588501578
XAC2423	XAC2423	IS1478 transposase	1.569132553
XAC3473	XAC3473	sensor histidine kinase	1.563105005
XAC1068	*stf*	phage-related tail protein	1.547879615
XAC2531	*btuB*	TonB-dependent receptor	1.52989273
XAC1338	XAC1338	oxidoreductase	1.526369207
XAC2966	XAC2966	hypothetical protein	1.486803374
XAC3133	*yggA*	membrane transport protein	1.479670175
XAC2141	*lytT*	two-component system regulatory protein	1.44212119
XAC3162	*bla*	beta lactamase	1.426624097
XAC2024	*cirA*	TonB-dependent receptor	1.415070068
XAC4218	*tatA*	twin-arginine translocation protein TatA	1.414369968
XAC0629	XAC0629	hypothetical protein	1.386558798
XAC3271	*tcp*	chemotaxis transducer	1.380089251
XAC1164	XAC1164	hypothetical protein	1.374341843
XAC1265	*hrpG*	HrpG protein	1.371008805

Table 3-11. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WXVM/AXVM
XAC0503	*nudC*	NADH pyrophosphatase	1.369464533
XAC2098	*syrE2*	ATP-dependent serine activating enzyme	1.361026084
XAC3731	*exsF*	regulatory protein	1.35746756
XAC3166	*bfeA*	ferric enterobactin receptor	1.339825933
XAC2014	XAC2014	TetR family transcriptional regulator	1.339547272
XAC1702	XAC1702	Mg-protoporphyrin IX monomethyl ester oxidative cyclase	1.339041029
XAC1680	XAC1680	serine protease	1.333363935
XAC1304	XAC1304	hypothetical protein	1.321989215
XAC3663	XAC3663	hypothetical protein	1.321799017
XAC3129	XAC3129	pseudouridylate synthase	1.32069952
XAC1137	*prpB*	2-methylisocitrate lyase	1.31928743
XAC2518	XAC2518	hypothetical protein	1.319238004
XAC3993	XAC3993	two-component system regulatory protein	1.314530614
XAC0151	XAC0151	hypothetical protein	1.302502741
XAC2589	*pheT*	phenylalanyl-tRNA synthetase subunit beta	1.300967384
XAC3505	*rhgB*	rhamnogalacturonase B	1.300716685
XAC3704	XAC3704	DNA polymerase-like protein	1.300562302
XAC1145	XAC1145	hypothetical protein	1.29968517
XAC2051	XAC2051	oxidoreductase	1.298496702
XAC3989	*prtI*	ECF sigma factor	1.298447877
XAC2537	XAC2537	peptidase	1.296382
XAC1172	XAC1172	hypothetical protein	1.295504189
XAC3814	*norM*	multidrug efflux protein	1.295412546
XAC3332	*cysH*	phosphoadenosine phosphosulfate reductase	1.294074263
XAC3255	XAC3255	hypothetical protein	1.290003478
XAC2430	XAC2430	Tn5044 transposase	1.286123031
XAC1388	XAC1388	hypothetical protein	1.28374325
XAC1266	*hrpXct*	HrpX protein	1.282732205
XAC2922	*hrpW*	HrpW protein	1.276470264
XAC2341	*gaa*	glutaryl-7-ACA acylase	1.274288431
XAC1713	XAC1713	carboxypeptidase-like protein	1.274030764
XAC3878	XAC3878	disulfide-isomerase	1.269624837
XAC3685	XAC3685	hypothetical protein	1.269583318
XAC2761	*xseB*	exodeoxyribonuclease VII small subunit	1.267073417
XAC4222	XAC4222	hypothetical protein	1.265839654
XAC3448	*btuB*	TonB-dependent receptor	1.263428471
XAC0501	XAC0501	hypothetical protein	1.261721563
XAC0435	*virK*	VirK protein	1.259483019
XAC2682	XAC2682	hypothetical protein	1.254711314

Table 3-11. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WXVM/AXVM
XAC2089	*kdsB*	3-deoxy-manno-octulosonate cytidylyltransferase	1.25383624
XAC0559	XAC0559	hypothetical protein	1.250733676
XAC0970	*tuf*	elongation factor Tu	1.249217448
XAC1284	XAC1284	two-component system regulatory protein	1.244543384
XAC0659	*mrdA*	penicillin-binding protein 2	1.242992158
XAC3909	*dpm1*	dolichol-phosphate mannosyltransferase	1.236551411
XAC3433	XAC3433	hypothetical protein	1.236157382
XAC1708	*exoD*	ExoD protein	1.233664964
XAC2529	*rhsD*	RhsD protein	1.232430714
XAC4314	*Y4JJ*	plasmid stability protein	1.231372873
XAC2863	XAC2863	hypothetical protein	1.230020862
XAC3923	*speA*	arginine decarboxylase	1.228248127
XAC0150	XAC0150	hypothetical protein	1.22725162
XAC1054	XAC1054	integrase	1.226369657
XAC0278	XAC0278	hypothetical protein	1.226297939
XAC1275	*xylB*	arabinosidase	1.22533929
XAC3432	XAC3432	serine/threonine protein kinase	1.224225852
XAC2123	XAC2123	hypothetical protein	1.223006639
XAC2960	XAC2960	hypothetical protein	1.221089262
XAC0382	*aspH*	aspartyl-asparaginyl beta-hydroxylase	1.220483682
XAC0186	XAC0186	hypothetical protein	1.22038462
XAC1479	XAC1479	OmpA family protein	1.218418475
XAC1356	XAC1356	hypothetical protein	1.218271514
XAC2955	XAC2955	nucleotidyl transferase	1.21712065
XAC2345	*argH*	argininosuccinate lyase	1.216795741
XAC1282	XAC1282	two-component system sensor protein	1.211033788
XAC4167	XAC4167	hypothetical protein	1.209260576
XAC3136	*exsG*	two-component system sensor protein	1.206808147
XAC3972	XAC3972	hypothetical protein	1.205604869
XAC1667	XAC1667	oxidoreductase	1.204126399
XAC2791	XAC2791	transcriptional regulator	1.204061868
XAC0543	XAC0543	hypothetical protein	1.203456667
XAC3465	*htrB*	lipid A biosynthesis lauroyl acyltransferase	1.202356052
XAC0003	*recF*	recombination protein F	1.201714859
XAC2824	XAC2824	phosphodiesterase-nucleotide pyrophosphatase	1.201122353
XAC0642	*rmrB*	MFS transporter	1.200503371
XAC3139	XAC3139	radical activating enzyme	1.197609691
XAC3850	*acrA*	acriflavin resistance protein	1.195210554
XAC2321	XAC2321	hydrolase	1.191152444

Table 3-11. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WXVM/AXVM
XAC3525	XAC3525	hypothetical protein	1.190776226
XAC2387	XAC2387	ribonuclease	1.189951672
XAC3430	*kch*	ion transporter	1.189829076
XAC3209	*ostB*	trehalose-6-phosphate phosphatase	1.189748354
XAC3590	XAC3590	oxidoreductase	1.189597864
XAC4073	XAC4073	flavodoxin	1.188859552
XAC2696	*nuoI*	NADH dehydrogenase subunit I	1.187130017
XAC1646	XAC1646	sulfite oxidase subunit YedZ	1.186877136
XAC2066	*acrD*	transporter	1.186361494
XAC4299	XAC4299	hypothetical protein	1.186326261
XAC2494	*yieO*	drug resistance translocase	1.186241703
XAC4371	XAC4371	polysaccharide deacetylase	1.185356334
XAC2087	*msbA*	ABC transporter ATP-binding protein	1.18438764
XAC2694	*nuoK*	NADH dehydrogenase subunit K	1.183675966
XAC2949	XAC2949	calcium-binding protein	1.182983913
XAC1473	XAC1473	hypothetical protein	1.181749396
XAC3044	XAC3044	hypothetical protein	1.18077869
XAC3067	*nudE*	ADP-ribose diphosphatase NudE	1.179854491
XAC4213	XAC4213	hypothetical protein	1.178728009
XAC3210	XAC3210	hypothetical protein	1.17756792
XAC3368	XAC3368	hypothetical protein	1.176869275
XAC3381	*pilQ*	fimbrial assembly protein	1.174243016
XAC3065	*mazG*	nucleoside triphosphate pyrophosphohydrolase	1.174000731
XAC3117	*pqqE*	pyrroloquinoline quinone biosynthesis protein PqqE	1.173512573
XAC3524	XAC3524	hypothetical protein	1.173210162
XAC0482	XAC0482	hypothetical protein	1.172459788
XAC3910	XAC3910	hypothetical protein	1.172342832
XAC0262	XAC0262	dipeptidyl anminopeptidase	1.172333892
XAC2692	*nuoM*	NADH dehydrogenase subunit M	1.171712976
XAC3684	XAC3684	hypothetical protein	1.170620368
XAC4151	*uvrD*	DNA-dependent helicase II	1.169730092
XAC1242	*pthX*	pathogenicity-like protein	1.169659036
XAC1281	*cheR*	chemotaxis protein	1.169622699
XAC1574	*pstB*	phosphate transporter ATP-binding protein	1.168429524
XAC3249	*colS*	two-component system sensor protein	1.167333473
XAC1764	XAC1764	regucalcin	1.167191817
XAC2086	*exbD*	biopolymer transport protein	1.167166771
XAC2129	*fabG*	3-ketoacyl-ACP reductase	1.16707058
XAC2312	XAC2312	hypothetical protein	1.166553432

Table 3-11. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WXVM/AXVM
XAC2693	*nuoL*	NADH dehydrogenase subunit L	1.166429198
XAC4204	XAC4204	hypothetical protein	1.166264863
XAC3476	*ybhD*	transcriptional regulator	1.166138952
XAC4080	*kefC*	glutathione-regulated potassium-efflux system protein	1.16570747
XAC2370	XAC2370	hypothetical protein	1.164762043
XAC1759	*gcvR*	glycine cleavage system transcriptional repressor	1.163986547
XAC2691	*nuoN*	NADH dehydrogenase subunit N	1.163834219
XAC2216	XAC2216	hypothetical protein	1.163338285
XAC0783	*ftsA*	cell division protein	1.163095132
XAC2908	*murD*	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	1.162467494
XAC0969	*fusA*	elongation factor G	1.16212181
XAC1283	XAC1283	two-component system sensor protein	1.160862998
XAC3490	XAC3490	amylosucrase or alpha amylase	1.159992676
XAC0025	XAC0025	hypothetical protein	1.15894674
XAC3521	*pncB*	nicotinate phosphoribosyltransferase	1.15811423
XAC3869	*bglX*	beta-glucosidase	1.157080382
XAC1516	*smpA*	hypothetical protein	1.157066171
XAC4031	*dinG*	ATP-dependent DNA helicase DinG	1.155715443
XAC0338	XAC0338	hypothetical protein	1.154658149
XAC2683	*pnp*	polynucleotide phosphorylase	1.15413532
XAC3796	XAC3796	lipopolysaccharide core biosynthesis glycosyl transferase	1.15356716
XAC3831	*rho*	transcription termination factor Rho	1.153021853
XAC1709	*tlyC*	hemolysin	1.151724215
XAC1598	XAC1598	hypothetical protein	1.150619899
XAC1317	*czcD*	cobalt-zinc-cadmium resistance protein	1.150495839
XAC3027	*emrA*	MFS transporter	1.149954036
XAC2591	*rplT*	50S ribosomal protein L20	1.149628506
XAC3157	*ycaD*	transmembrane transport protein	1.149427325
XAC0866	*ostA*	organic solvent tolerance protein	1.148219572
XAC3833	*ampR*	B-lactamase regulatory protein	1.147739985
XAC2745	XAC2745	metallopeptidase	1.147535942
XAC2019	XAC2019	hypothetical protein	1.147242156
XAC1303	*mutS*	DNA mismatch repair protein MutS	1.147138182
XAC2713	XAC2713	oxidoreductase	1.146816656
XAC3326	*acrF*	acriflavin resistance protein	1.14569161
XAC3110	XAC3110	glycosyltransferase	1.144788182
XAC0234	XAC0234	hypothetical protein	1.144776321

Table 3-11. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WXVM/AXVM
XAC2909	XAC2909	hypothetical protein	1.144705443
XAC4022	*phoQ*	two-component system sensor protein	1.142982308
XAC2695	*nuoJ*	NADH dehydrogenase subunit J	1.142236467
XAC3487	*cebR*	transcriptional regulator	1.139698561
XAC0944	*prfA*	peptide chain release factor 1	1.139120231
XAC2136	XAC2136	oxidoreductase	1.138862248
XAC3256	*xrvA*	virulence regulator	1.1380708
XAC2939	XAC2939	acetyltransferase	1.137692915
XAC0476	*trpE*	anthranilate synthase component I	1.137278346
XAC1840	XAC1840	methylthioribulose-1-phosphate dehydratase	1.136880376
XAC2593	*infC*	translation initiation factor IF-3	1.13648463
XAC0997	*rplQ*	50S ribosomal protein L17	1.134702641
XAC1262	XAC1262	hypothetical protein	1.132558868
XAC0993	*rpsM*	30S ribosomal protein S13	1.131512264
XAC1420	XAC1420	hypothetical protein	1.131435935
XAC2054	XAC2054	two-component system sensor protein	1.13113872
XAC1629	XAC1629	hypothetical protein	1.130969214
XAC3671	XAC3671	nucleotide-binding protein	1.129892218
XAC2419	*traY*	hypothetical protein	1.129882215
XAC2388	XAC2388	hypothetical protein	1.129166509
XAC2728	*psd*	phosphatidylserine decarboxylase	1.128263004
XAC2697	*nuoH*	NADH dehydrogenase subunit H	1.127908194
XAC2825	*yadQ*	chloride channel	1.127738383
XAC2020	XAC2020	hypothetical protein	1.127625306
XAC2969	XAC2969	hypothetical protein	1.127114885
XAC0772	*mraW*	S-adenosyl-methyltransferase MraW	1.126654813
XAC3788	*rpoD*	RNA polymerase sigma factor RpoD	1.12527377
XAC2700	*nuoE*	NADH dehydrogenase subunit E	1.125103265
XAC2999	XAC2999	peptidase	1.124990925
XAC3343	XAC3343	hypothetical protein	1.124350982
XAC2768	XAC2768	hypothetical protein	1.123518953
XAC3142	*tolB*	translocation protein TolB	1.123326621
XAC4074	*nrdF*	ribonucleotide-diphosphate reductase subunit beta	1.12192923
XAC3846	*queF*	7-cyano-7-deazaguanine reductase	1.121473748
XAC0431	*glgX*	glycogen debranching protein	1.119466319
XAC3971	XAC3971	hypothetical protein	1.118465117
XAC3462	*pcm*	L-isoaspartate protein carboxylmethyltransferase	1.116530484
XAC4069	XAC4069	hypothetical protein	1.116032713
XAC0683	XAC0683	two-component system sensor protein	1.115904391

Table 3-11. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WXVM/AXVM
XAC3589	XAC3589	hypothetical protein	1.115465221
XAC4004	XAC4004	peptidase	1.115410915
XAC0937	*rbn*	ribonuclease BN/unknown domain fusion protein	1.114810365
XAC3386	*mrcA*	penicillin-binding protein 1A	1.114091738
XAC0771	XAC0771	cell division protein MraZ	1.113965299
XAC3037	XAC3037	hydrolase	1.113581621
XAC2010	XAC2010	recombination factor protein RarA	1.113459243
XAC0630	*aspC*	hypothetical protein	1.112720509
XAC4336	*recB*	exodeoxyribonuclease V subunit beta	1.112518148
XAC0547	XAC0547	hypothetical protein	1.111918403
XAC0144	*iroN*	TonB-dependent receptor	1.11172718
XAC3464	*kdtA*	3-deoxy-D-manno-octulosonic-acid transferase	1.11084302
XAC0779	*murG*	undecaprenyldiphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase	1.110547021
XAC2320	XAC2320	glutamine cyclotransferase	1.109835366
XAC0731	XAC0731	hypothetical protein	1.109562937
XAC1098	*moaC*	molybdenum cofactor biosynthesis protein MoaC	1.109033631
XAC3986	XAC3986	hydrolase	1.108097884
XAC2420	XAC2420	hypothetical protein	1.104359751
XAC3877	XAC3877	hypothetical protein	1.102492558
XAC4081	*zwf*	glucose-6-phosphate 1-dehydrogenase	1.102461044
XAC0280	XAC0280	ATPase	1.102358355
XAC0678	XAC0678	hypothetical protein	1.102022482
XAC1428	*map*	methionine aminopeptidase	1.101376515
XAC2625	*uvrB*	excinuclease ABC subunit B	1.100974109
XAC3561	*slt*	soluble lytic murein transglycosylase	1.100402961
XAC1783	*pcnB*	polynucleotide adenylyltransferase	1.100281171
XAC0785	*lpxC*	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase	1.098602988
XAC0774	*ftsI*	penicillin-binding protein 3	1.098434576
XAC0992	*secY*	preprotein translocase subunit SecY	1.098276194
XAC2614	*virB4*	VirB4 protein	1.098231486
XAC2980	*mgtE*	Mg++ transporter	1.098077476
XAC2763	XAC2763	extracellular protease	1.097871294
XAC1716	*pyrG*	CTP synthetase	1.097814907
XAC2701	*nuoD*	NADH dehydrogenase subunit D	1.096469297
XAC2389	*uup*	ABC transporter ATP-binding protein	1.096419143

Table 3-11. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WXVM/AXVM
XAC4286	*mutM*	formamidopyrimidine-DNA glycosylase	1.09597188
XAC2332	*metX*	homoserine O-acetyltransferase	1.094871985
XAC2857	XAC2857	hypothetical protein	1.09458925
XAC1224	*minE*	cell division topological specificity factor MinE	1.094411718
XAC2698	*nuoG*	NADH dehydrogenase subunit G	1.093780318
XAC3393	*spoT*	pentaphosphate guanosine-3'-pyrophosphohydrolase	1.093113891
XAC1408	*lpxB*	lipid-A-disaccharide synthase	1.092309972
XAC2377	*ubiG*	3-demethylubiquinone-9 3-methyltransferase	1.092111048
XAC3463	*tolC*	TolC protein	1.092037251
XAC0842	*thyA*	thymidylate synthase	1.091613283
XAC0626	XAC0626	outer membrane lipoprotein	1.09029366
XAC3408	XAC3408	cell division protein ZapA	1.089478643
XAC0631	*ptrB*	oligopeptidase B	1.088612894
XAC0865	*surA*	peptidyl-prolyl cis-trans isomerase	1.088038778
XAC1089	*rnhA*	ribonuclease H	1.087115047
XAC0728	*gsh1*	glutamate-cysteine ligase	1.086568153
XAC1459	*msbA*	ABC transporter ATP-binding protein	1.083506505
XAC1911	XAC1911	hypothetical protein	1.080916219
XAC2911	*lysA*	bifunctional aspartate kinase/diaminopimelate decarboxylase	1.08036793
XAC2428	XAC2428	hypothetical protein	1.078949686
XAC4075	*nrdA*	ribonucleotide-diphosphate reductase subunit alpha	1.078866378
XAC0667	*lipB*	lipoate-protein ligase B	1.078675178
XAC2551	XAC2551	transcriptional regulator	1.0778973
XAC0307	XAC0307	nucleoside hydrolase	1.077279637
XAC4308	*kgtP*	dicarboxylate transport protein	1.077142988
XAC3140	XAC3140	hypothetical protein	1.075377886
XAC3141	*ompP6*	outer membrane protein P6	1.074840946
XAC3145	*tolQ*	TolQ protein	1.073781118
XAC2699	*nuoF*	NADH-ubiquinone oxidoreductase NQO1 subunit	1.073471956
XAC2717	*trpB*	tryptophan synthase subunit beta	1.072698883
XAC0466	XAC0466	lytic enzyme	1.072323551
XAC0987	*rplF*	50S ribosomal protein L6	1.06960567
XAC0499	XAC0499	iron-sulfur cluster insertion protein ErpA	1.068741717
XAC2092	*uvrC*	excinuclease ABC subunit C	1.068561306
XAC0926	XAC0926	hypothetical protein	1.068110137

Table 3-11. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WXVM/AXVM
XAC0664	*dacC*	penicillin-binding protein 6	1.066853695
XAC2053	*tex*	transcription-like protein	1.063685457
XAC1863	*greA*	transcription elongation factor GreA	1.063135125
XAC4045	XAC4045	hypothetical protein	1.062585408
XAC3851	XAC3851	hypothetical protein	1.061810454
XAC0979	*rplP*	50S ribosomal protein L16	1.061276053
XAC2530	XAC2530	hypothetical protein	1.059660422
XAC1739	*lexA*	LexA repressor	1.057997036
XAC0978	*rpsC*	30S ribosomal protein S3	1.055386761
XAC1876	*lysS*	lysyl-tRNA synthetase	1.055250006
XAC2386	*sodM*	superoxidase dismutase	1.054560193
XAC3352	*gapA*	glyceraldehyde-3-phosphate dehydrogenase	1.053735208
XAC3575	*etf-QO*	flavoprotein-ubiquinone oxidoreductase	1.052411511
XAC2293	XAC2293	dehydratase	1.049383309
XAC1653	*serS*	seryl-tRNA synthetase	1.048983343
XAC4023	*phoP*	two-component system regulatory protein	1.04705924
XAC2077	*sdhA*	succinate dehydrogenase flavoprotein subunit	1.037306851
XAC1348	*atoB*	acetoacetyl-CoA thiolase	1.036315471
XAC2767	*tldD*	TldD protein	1.031928917
XAC3804	*smg*	hypothetical protein	1.031496226
Underexpressed Genes			
XAC3260	*mobL*	plasmid mobilization protein	-15.87890005
XAC3594	XAC3594	hypothetical protein	-3.566676924
XAC3298	XAC3298	integrase	-3.49094522
XAC4225	*xylA*	xylose isomerase	-3.361963503
XAC3593	XAC3593	NAD dependent epimerase/dehydratase/dehydrogenase	-3.088200659
XAC1492	XAC1492	hypothetical protein	-2.634526927
XAC1507	*mobL*	plasmid mobilization protein	-2.109894919
XAC0210	*sodC2*	superoxide dismutase	-2.035026214
XAC0029	*egl*	cellulase	-1.932933038
XAC0753	XAC0753	hypothetical protein	-1.92587448
XAC0540	XAC0540	ribonuclease	-1.911336018
XAC0757	*kdpB*	potassium-transporting ATPase subunit B	-1.838565045
XAC2151	*yapH*	YapH protein	-1.800840033
XAC3323	XAC3323	hypothetical protein	-1.774277616
XAC0758	*kdpC*	potassium-transporting ATPase subunit C	-1.718279526
XAC3273	XAC3273	histidine kinase-response regulator hybrid protein	-1.699159905

Table 3-11. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WXVM/AXVM
XAC0048	XAC0048	hypothetical protein	-1.562063358
XAC2598	XAC2598	hypothetical protein	-1.540700322
XAC3364	XAC3364	hypothetical protein	-1.51892591
XAC0028	*egl*	cellulase	-1.490864678
XAC0759	*kdpD*	two-component system sensor protein	-1.48694649
XAC1034	XAC1034	peptidyl-Asp metalloendopeptidase	-1.464826868
XAC2364	*eutP*	ethanolamin permease	-1.438426647
XAC1927	*aslB*	Fe-S oxidoreductase	-1.418653513
XAC4176	*actP*	acetate permease	-1.415778246
XAC0957	*tuf*	elongation factor Tu	-1.415709759
XAC0051	*asnB*	asparagine synthase	-1.39410184
XAC0465	XAC0465	metalloproteinase	-1.382134115
XAC0612	*engXCA*	cellulase	-1.377531737
XAC0346	XAC0346	degenerated cellulase	-1.364460865
XAC2596	*cgt*	cyclomaltodextrin glucanotransferase	-1.356697125
XAC3300	*estA*	esterase	-1.354623982
XAC2600	*btuB*	TonB-dependent receptor	-1.341832005
XAC0928	XAC0928	extracellular protease	-1.337385083
XAC0235	*dhaA*	haloalkane dehalogenase	-1.334338186
XAC1821	*thrB*	homoserine kinase	-1.325952422
XAC2992	XAC2992	endoproteinase ArgC	-1.32122178
XAC2185	*fhuA*	ferrichrome-iron receptor	-1.316586488
XAC0122	*tldD*	TldD protein	-1.306361106
XAC2830	*fhuA*	TonB-dependent receptor	-1.303722242
XAC4052	XAC4052	TonB-like protein	-1.298382438
XAC0190	XAC0190	hypothetical protein	-1.297342465
XAC0202	XAC0202	hypothetical protein	-1.296144882
XAC3460	XAC3460	hypothetical protein	-1.284270434
XAC1321	*mucD*	periplasmic protease	-1.274712139
XAC1011	XAC1011	oxidoreductase	-1.274101511
XAC3868	*ylil*	dehydrogenase	-1.269934908
XAC0067	*mdpB*	microcystin dependent protein	-1.265598564
XAC4177	XAC4177	hypothetical protein	-1.265503813
XAC2597	*suc1*	transporter	-1.263762776
XAC0082	XAC0082	short chain oxidoreductase	-1.259455421
XAC3768	XAC3768	chemotaxis protein	-1.251708156
XAC3471	*dctA*	C4-dicarboxylate transporter DctA	-1.247329501
XAC0445	*pdhB*	pyruvate dehydrogenase E1 beta subunit	-1.237752177
XAC4172	XAC4172	transcriptional regulator	-1.236557695
XAC3457	*leuD*	isopropylmalate isomerase small subunit	-1.236156678
XAC2152	XAC2152	hypothetical protein	-1.230846451

Table 3-11. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WXVM/AXVM
XAC1978	*flgJ*	flagellar rod assembly protein/muramidase	-1.229827393
XAC1021	XAC1021	hypothetical protein	-1.222576427
XAC3458	*leuC*	isopropylmalate isomerase large subunit	-1.221916323
XAC4271	XAC4271	hypothetical protein	-1.221079227
XAC1951	*fliI*	flagellar protein	-1.219981228
XAC1655	XAC1655	transcriptional regulator	-1.217452535
XAC1287	*galM*	aldose 1-epimerase	-1.216073189
XAC1107	XAC1107	integrase	-1.207377939
XAC3715	XAC3715	hypothetical protein	-1.204304527
XAC0495	XAC0495	two-component system regulatory protein	-1.201603841
XAC2489	XAC2489	beta alanine--pyruvate transaminase	-1.199071845
XAC2984	XAC2984	peptidase	-1.196915053
XAC0129	*aldA*	chloroacetaldehyde dehydrogenase	-1.193030153
XAC1344	XAC1344	hypothetical protein	-1.186168829
XAC3887	*ctaD*	cytochrome C oxidase subunit I	-1.185672826
XAC1983	*flgE*	flagellar hook protein FlgE	-1.183647066
XAC2878	XAC2878	hypothetical protein	-1.180721753
XAC0229	XAC0229	MFS transporter	-1.18039229
XAC0112	XAC0112	hypothetical protein	-1.178652766
XAC0360	*glpD*	glycerol-3-phosphate dehydrogenase	-1.175973943
XAC0498	XAC0498	hypothetical protein	-1.174623036
XAC3449	*tar*	chemotaxis protein	-1.171887227
XAC3454	*tdcB*	threonine dehydratase	-1.171761775
XAC0609	XAC0609	zinc protease	-1.16848306
XAC1910	*cirA*	TonB-dependent receptor	-1.168184575
XAC3514	XAC3514	serine protease	-1.165623897
XAC0189	*iorA*	indolepyruvate ferredoxin oxidoreductase	-1.164978089
XAC3459	XAC3459	LysR family transcriptional regulator	-1.160188916
XAC1427	*pru*	protein U	-1.156274735
XAC3888	*ctaC*	cytochrome C oxidase subunit II	-1.151497442
XAC2192	XAC2192	two-component system sensor protein	-1.150089667
XAC0226	*ntrC*	two-component system regulatory protein	-1.145996196
XAC1462	*cynT*	carbonic anhydrase	-1.142486217
XAC0287	XAC0287	quinone oxidoreductase	-1.141434126
XAC2764	XAC2764	hypothetical protein	-1.141007932
XAC3609	*uptA*	fumarylacetoacetate hydrolase	-1.140044507
XAC0312	XAC0312	LysR family transcriptional regulator	-1.133991752
XAC1519	*recN*	recombination protein N	-1.128044065
XAC3450	*ggt*	gamma-glutamyltranspeptidase	-1.125846979
XAC1245	XAC1245	hypothetical protein	-1.125528858
XAC0254	*yjl094C*	Na+/H+-exchanging protein	-1.120567337

Table 3-11. Continued.

Locus tag	*Gene Name*	Product	Log2 Fold Change WXVM/AXVM
XAC2103	XAC2103	DNA recombinase	-1.119674933
XAC3998	XAC3998	hypothetical protein	-1.118410688
XAC0224	*poxB*	pyruvate dehydrogenase	-1.118133461
XAC2503	*fruA*	PTS system fructose-specific transporter subunit IIBC	-1.118046447
XAC1981	*flgG*	flagellar basal body rod protein FlgG	-1.118019747
XAC1537	XAC1537	hypothetical protein	-1.114259852
XAC0032	*gltD*	glutamate synthase subunit beta	-1.111298379
XAC0033	*gltB*	glutamate synthase subunit alpha	-1.10933767
XAC3313	*susB*	alpha-glucosidase	-1.108486383
XAC3309	XAC3309	aminopeptidase	-1.103704073
XAC0868	XAC0868	hypothetical protein	-1.103538809
XAC3268	XAC3268	hypothetical protein	-1.103342387
XAC4305	*fusE*	fusaric acid resistance protein	-1.102472813
XAC4179	*acs*	acetyl-CoA synthetase	-1.102184211
XAC1521	*grpE*	heat shock protein GrpE	-1.101307396
XAC3239	*pilB*	pilus biogenesis protein	-1.100613941
XAC3739	XAC3739	hypothetical protein	-1.09922564
XAC2881	*cstA*	carbon starvation protein A	-1.094372426
XAC0555	XAC0555	hypothetical protein	-1.093597616
XAC3959	XAC3959	hypothetical protein	-1.092511912
XAC3652	*atpH*	ATP synthase F0F1 subunit delta	-1.091840333
XAC4342	*yrbC*	toluene tolerance protein	-1.09134779
XAC3889	XAC3889	hypothetical protein	-1.089138387
XAC2758	*gltT*	glutamate symporter	-1.082657383
XAC1254	*ileS*	isoleucyl-tRNA synthetase	-1.072014975
XAC0887	XAC0887	gluconolactonase	-1.071876929
XAC2068	*edd*	phosphogluconate dehydratase	-1.069481164
XAC1123	*fabH*	3-oxoacyl-ACP synthase	-1.067882822
XAC3626	XAC3626	hypothetical protein	-1.066740144
XAC2723	*asd*	aspartate-semialdehyde dehydrogenase	-1.060890441
XAC1047	XAC1047	hypothetical protein	-1.044681666
XAC2872	XAC2872	metallopeptidase	-1.041077088
XAC3236	*sucC*	succinyl-CoA synthetase subunit beta	-1.02693388

Table 3-12. Differentially expressed genes shared between strains *X. citri* subsp. *citri* str. 306 (A) and *X. citri* subsp. *citri* str. A^w 12879 (W) in both NB (nutrient rich) medium and XVM2 (hrp inducing) medium. Cut off value of Log2 fold Change ≥ 1 for overexpressed genes and ≤ -1 for underexpressed genes. Log2 fold change 1 (|fold change| 2). Locus tag of only strain A is used for ease of comparison.

Locus tag	Gene Name	Product	Log_2 Fold Change WNB/ANB	Log_2 Fold Change WXVM/ AXVM
Overexpressed Genes				
XAC4248	*gnl*	gluconolactonase	10.5068058	3.87777102
XAC3474	*cit1*	citrate carrier protein	2.51470588	1.73848736
XAC3445	XAC3445	transcriptional regulator	2.49930759	1.79169953
XAC3475	XAC3475	hypothetical protein	2.48547363	1.82847725
XAC0338	XAC0338	hypothetical protein	2.27694386	1.15465815
XAC1338	XAC1338	oxidoreductase	2.17477308	1.52636921
XAC3712	XAC3712	metallopeptidase	1.987497	1.81512161
XAC0999	*cirA*	colicin I receptor	1.92756557	1.7665622
XAC3229	*orfT*	cointegrate resolution protein T	1.92060358	1.53265533
XAC2520	XAC2520	TonB-dependent receptor	1.80398105	1.79888096
XAC2538	XAC2538	hypothetical protein	1.75327701	2.09328733
XAC2860	XAC2860	hypothetical protein	1.73979456	2.03864784
XAC3444	*btuB*	TonB-dependent receptor	1.7377432	2.6269591
XAC1574	*pstB*	phosphate transporter ATP-binding protein	1.69310228	1.16842952
XAC2763	XAC2763	extracellular protease	1.57265135	1.09787129
XAC1266	*hrpXct*	HrpX protein	1.56826769	1.2827322
XAC1164	XAC1164	hypothetical protein	1.51586288	1.37434184
XAC2604	XAC2604	ISxac4 transposase	1.49617037	1.95536706
XAC3028	XAC3028	histidine kinase-response regulator hybrid protein	1.49594471	1.59404501
XAC2141	*lytT*	two-component system regulatory protein	1.48741781	1.44212119
XAC3490	XAC3490	amylosucrase or alpha amylase	1.47448013	1.15999268
XAC1137	*prpB*	2-methylisocitrate lyase	1.4552837	1.31928743
XAC2423	XAC2423	IS1478 transposase	1.38969694	1.56913255
XAC3473	XAC3473	sensor histidine kinase	1.3669144	1.56310501

Table 3-12. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change WNB/ANB	Log2 Fold Change WXVM/ AXVM
XAC3704	XAC3704	DNA polymerase-like protein	1.35335496	1.3005623
XAC3381	*pilQ*	fimbrial assembly protein	1.30821059	1.17424302
XAC2537	XAC2537	peptidase	1.30633822	1.296382
XAC1068	*stf*	phage-related tail protein	1.2928669	1.54787962
XAC0970	*tuf*	elongation factor Tu	1.24189979	1.24921745
XAC1265	*hrpG*	HrpG protein	1.19458611	1.3710088
XAC2949	XAC2949	calcium-binding protein	1.19212609	1.18298391
XAC2312	XAC2312	hypothetical protein	1.18549243	1.16655343
XAC3166	*bfeA*	ferric enterobactin receptor	1.18429865	1.33982593
XAC3433	XAC3433	hypothetical protein	1.1770105	1.23615738
XAC2589	*pheT*	phenylalanyl-tRNA synthetase subunit beta	1.1717389	1.30096738
XAC2341	*gaa*	glutaryl-7-ACA acylase	1.17093649	1.27428843
XAC3909	*dpm1*	dolichol-phosphate mannosyltransferase	1.15560875	1.23655141
XAC0025	XAC0025	hypothetical protein	1.15163323	1.15894674
XAC3505	*rhgB*	rhamnogalacturonase B	1.15135909	1.30071669
XAC3923	*speA*	arginine decarboxylase	1.14910526	1.22824813
XAC3476	*ybhD*	transcriptional regulator	1.14537171	1.16613895
XAC2531	*btuB*	TonB-dependent receptor	1.13855221	1.52989273
XAC2824	XAC2824	phosphodiesterase-nucleotide pyrophosphatase	1.13112145	1.20112235
XAC3851	XAC3851	hypothetical protein	1.13017888	1.06181045
XAC2530	XAC2530	hypothetical protein	1.12768757	1.05966042
XAC2019	XAC2019	hypothetical protein	1.12440991	1.14724216
XAC3850	*acrA*	acriflavin resistance protein	1.12188969	1.19521055
XAC3465	*htrB*	lipid A biosynthesis lauroyl acyltransferase	1.11670532	1.20235605
XAC0969	*fusA*	elongation factor G	1.10928452	1.16212181
XAC3463	*tolC*	TolC protein	1.10590689	1.09203725
XAC2717	*trpB*	tryptophan synthase subunit beta	1.10272748	1.07269888
XAC3869	*bglX*	beta-glucosidase	1.10150678	1.15708038

Table 3-12. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change WNB/ANB	Log2 Fold Change WXVM/ AXVM
XAC0731	XAC0731	hypothetical protein	1.0998918	1.10956294
XAC1713	XAC1713	carboxypeptidase-like protein	1.09542185	1.27403076
XAC3487	*cebR*	transcriptional regulator	1.09378434	1.13969856
XAC2908	*murD*	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	1.09290591	1.16246749
XAC2999	XAC2999	peptidase	1.09210033	1.12499093
XAC1716	*pyrG*	CTP synthetase	1.08983099	1.09781491
XAC2020	XAC2020	hypothetical protein	1.08903328	1.12762531
XAC2345	*argH*	argininosuccinate lyase	1.08902751	1.21679574
XAC3326	*acrF*	acriflavin resistance protein	1.08606097	1.14569161
XAC4299	XAC4299	hypothetical protein	1.08569401	1.18632626
XAC2745	XAC2745	metallopeptidase	1.08291429	1.14753594
XAC4022	*phoQ*	two-component system sensor protein	1.08145677	1.14298231
XAC3352	*gapA*	glyceraldehyde-3-phosphate dehydrogenase	1.08013231	1.05373521
XAC2691	*nuoN*	NADH dehydrogenase subunit N	1.07828665	1.16383422
XAC2713	XAC2713	oxidoreductase	1.07779892	1.14681666
XAC1262	XAC1262	hypothetical protein	1.07765163	1.13255887
XAC1282	XAC1282	two-component system sensor protein	1.07661874	1.21103379
XAC0944	*prfA*	peptide chain release factor 1	1.07246641	1.13912023
XAC2980	*mgtE*	Mg++ transporter	1.07030448	1.09807748
XAC4004	XAC4004	peptidase	1.06919341	1.11541091
XAC2909	XAC2909	hypothetical protein	1.06746918	1.14470544
XAC4023	*phoP*	two-component system regulatory protein	1.06644043	1.04705924
XAC3671	XAC3671	nucleotide-binding protein	1.06558108	1.12989222
XAC3145	*tolQ*	TolQ protein	1.06518531	1.07378112
XAC2053	*tex*	transcription-like protein	1.06335497	1.06368546
XAC1428	*map*	methionine aminopeptidase	1.06164489	1.10137652
XAC3110	XAC3110	glycosyltransferase	1.05390208	1.14478818

Table 3-12. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change WNB/ANB	Log2 Fold Change WXVM/ AXVM
XAC0382	*aspH*	aspartyl-asparaginyl beta-hydroxylase	1.05384546	1.22048368
XAC3141	*ompP6*	outer membrane protein P6	1.05357499	1.07484095
XAC2692	*nuoM*	NADH dehydrogenase subunit M	1.05340355	1.17171298
XAC2683	*pnp*	polynucleotide phosphorylase	1.0532156	1.15413532
XAC2698	*nuoG*	NADH dehydrogenase subunit G	1.05135027	1.09378032
XAC2701	*nuoD*	NADH dehydrogenase subunit D	1.04909217	1.0964693
XAC2700	*nuoE*	NADH dehydrogenase subunit E	1.04716601	1.12510326
XAC3393	*spoT*	pentaphosphate guanosine-3'-pyrophosphohydrolase	1.04097272	1.09311389
Underexpressed Genes				
XAC1492	XAC1492	hypothetical protein	-10.21684841	-2.634526927
XAC3594	XAC3594	hypothetical protein	-7.376270824	-3.566676924
XAC3593	XAC3593	NAD dependent epimerase/dehydratase/dehydrogenase	-6.332084605	-3.088200659
XAC3291	XAC3291	hypothetical protein	-3.922051423	-2.181287589
XAC1507	*mobL*	plasmid mobilization protein	-3.281412651	-2.109894919
XAC0048	XAC0048	hypothetical protein	-3.031674789	-1.562063358
XAC3273	XAC3273	histidine kinase-response regulator hybrid protein	-2.657889437	-1.699159905
XAC2636	XAC2636	hypothetical protein	-2.452389885	-2.843259344
XAC3323	XAC3323	hypothetical protein	-2.235229866	-1.774277616
XAC0029	*egl*	cellulase	-2.139857262	-1.932933038
XAC0028	*egl*	cellulase	-2.12773726	-1.490864678
XAC0094	XAC0094	hypothetical protein	-1.966999614	-1.591122466
XAC0210	*sodC2*	superoxide dismutase	-1.915635997	-2.035026214
XAC0201	*adh*	alcohol dehydrogenase	-1.802254241	-2.387744174
XAC2597	*suc1*	transporter	-1.613187569	-1.263762776
XAC0051	*asnB*	asparagine synthase	-1.569680723	-1.39410184
XAC2600	*btuB*	TonB-dependent receptor	-1.522112982	-1.341832005
XAC0235	*dhaA*	haloalkane dehalogenase	-1.483694734	-1.334338186

Table 3-12. Continued.

Locus tag	Gene Name	Product	Log2 Fold Change WNB/ANB	Log2 Fold Change WXVM/ AXVM
XAC3364	XAC3364	hypothetical protein	-1.470066015	-1.51892591
XAC2598	XAC2598	hypothetical protein	-1.468776033	-1.540700322
XAC0957	*tuf*	elongation factor Tu	-1.376878728	-1.415709759
XAC0540	XAC0540	ribonuclease	-1.372944605	-1.911336018
XAC3300	*estA*	esterase	-1.351414413	-1.354623982
XAC2596	*cgt*	cyclomaltodextrin glucanotransferase	-1.344697177	-1.356697125
XAC1034	XAC1034	peptidyl-Asp metalloendopeptidase	-1.33483264	-1.464826868
XAC0422	XAC0422	ABC transporter substrate-binding protein	-1.276605046	-1.199270308
XAC3739	XAC3739	hypothetical protein	-1.263549906	-1.09922564
XAC0612	*engXCA*	cellulase	-1.19584223	-1.377531737
XAC2103	XAC2103	DNA recombinase	-1.170631164	-1.119674933
XAC1321	*mucD*	periplasmic protease	-1.165075851	-1.274712139
XAC1521	*grpE*	heat shock protein GrpE	-1.161281031	-1.101307396
XAC0465	XAC0465	metalloproteinase	-1.159209175	-1.382134115
XAC0189	*iorA*	indolepyruvate ferredoxin oxidoreductase	-1.139472125	-1.164978089
XAC0224	*poxB*	pyruvate dehydrogenase	-1.135612256	-1.118133461
XAC3458	*leuC*	isopropylmalate isomerase large subunit	-1.122307106	-1.221916323
XAC0495	XAC0495	two-component system regulatory protein	-1.118411592	-1.201603841
XAC2151	*yapH*	YapH protein	-1.113301035	-1.800840033
XAC2992	XAC2992	endoproteinase ArgC	-1.102233164	-1.32122178
XAC0122	*tldD*	TldD protein	-1.101179671	-1.306361106

Table 3-13. Differential expression of effctor genes shared between strains *X. citri* subsp. *citri* str. 306 (A) and *X. citri* subsp. *citri* str. A^w 12879 (W) in both NB (nutrient rich) medium and XVM2 (hrp inducing) medium. FDR values are in parenthesis. The ones that pass cut-off value of 0.05 are marked in green

Effector class	Xcaw	XccA	Log_2 fold change(FDR) WNB/ANB	Log_2 fold change (FDR) WXVM/AXVM	Promoter region
AvrBs2	XCAW_00465	XAC0076	1.04 (0.72)	1.2 (0.08)	Different -10
XopA (Hpa1/HpaG)	XCAW_00826	XAC0416	1.48 (0.53)	1.14 (0.16)	Different -35
XopE1 (AvrXacE1)	XCAW_00686	XAC0286	-1.03 (0.77)	1.19 (0.06)	Same
XopE3 (AvrXacE2)	XCAW_03515	XAC3224	1.01 (1.0)	1.04 (0.38)	Same
XopF2	XCAW_01388 Ψ	XAC2785 Ψ	1.21 (0.21)	2.39 (0.19)	Same
XopI	XCAW_03828	XAC0754	1.74 (0.06)	1.15 (0.46)	Same
XopK	XCAW_03372	XAC3085	1.23 (0.48)	1.04 (0.83)	Same
XopL	XCAW_03376	XAC3090	1.04 (0.82)	1.59 (0.03)*	Same
XopQ	XCAW_04706	XAC4333	1.05 (0.72)	-1.07 (0.19)	Same
XopR	XCAW_00677	XAC0277	-1.01 (1.0)	1.23 (0.14)	Same
XopV	XCAW_03980	XAC0601	1.17 (0.21)	1.43 (0.08)	Same
XopX	XCAW_00956	XAC0543	1.20 (0.20)	1.20 (0.05)*	Same
XopZ1	XCAW_01815	XAC2009	1.03 (0.85)	1.16 (0.09)	Same
XopAD	XCAW_00082	XAC4213	1.03 (0.87)	1.18 (0.02)*	Same
XopAI	XCAW_01099	XAC3230	-1.02 (0.85)	1.09 (0.10)	Same
XopAK	XCAW_04369	XAC3666	-1.01 (0.96)	1.13 (0.12)	Same
XopAP	XCAW_03269	XAC2990	-1.07 (0.04)*	1.01 (1.0)	Same
HpaA	XCAW_00810	XAC0400	-7.31 (0.26)	1.07 (0.81)	Same
HrpW(PopW)	XCAW_03200	XAC2922	1.03 (0.60)	1.28 (0.02)*	Same
XopAQ	XCAW_03514	No annotation between XAC3223 and XAC3224	-1.02 (0.81)	1.41 (0.04)*	Same
XopE2 (AvrXacE3, AvrXccE1)	XCAW_03520	XACb0011	-1.00 (1.0)	-1.02 (0.56)	Different -35
XopN	XCAW_01387	XAC2786	1.23 (0.16)	1.40 (0.06)	Same
XopP	XCAW_01310	XAC1208	1.06 (0.36)	1.16 (0.10)	Same
XopAE (HpaF/HpaG)	XCAW_00801	XAC0393	-1.10 (0.69)	1.04 (0.56)	Same
XopC2	XCAW_01311Ψ	XAC1209Ψ XAC1210Ψ	-- -1.02 (0.99)	-- 1.04 (0.64)	Same

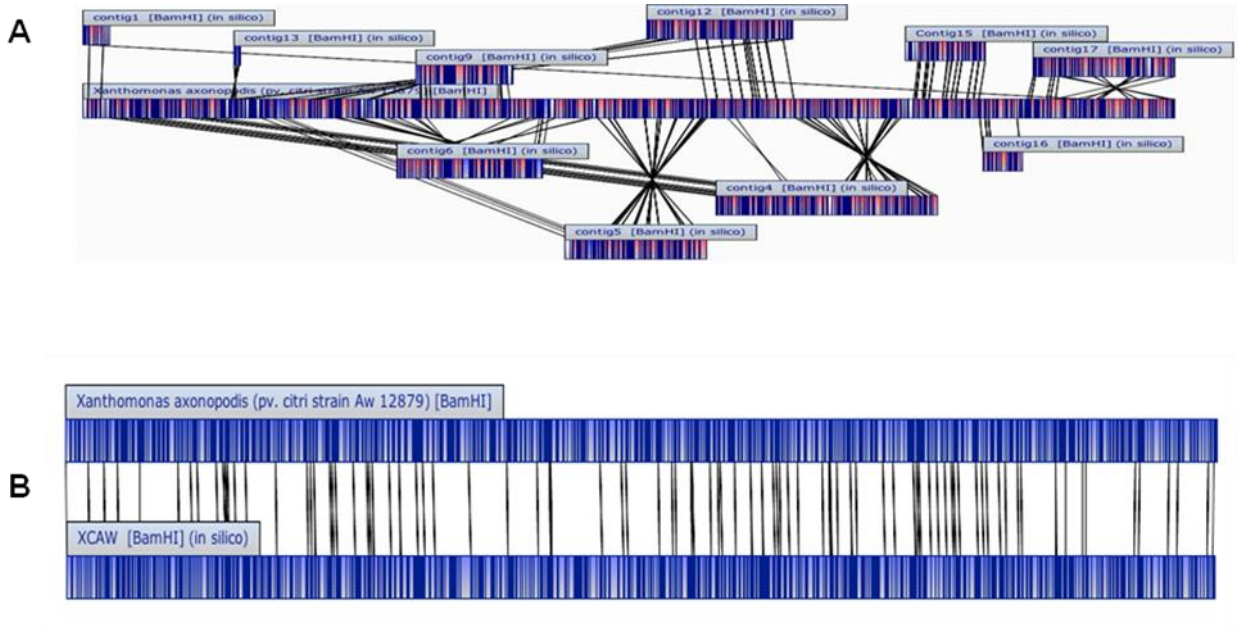


Figure 3-1. Alignments between the whole-genome optical maps and the in silico genome sequence assemblies at various stages of the project. Dark blue represents cut sites, light blue regions indicate alignment, white regions indicate no alignment. Alignment lines for inversions and translocations are highlighted in pink. Panel A: An early comparison of the optical map derived from *Bam*H1 digestion of the *X. citri* subsp. *citri* A^{w} 12879 (Xcaw) genome to the assembled scaffolds generated by traditional sequencing technologies. The Xcaw optical map derived from BamH1 digestion of the chromosome is presented as a single contig in the center. The sequenced genome contains 17 scaffolds of which 10 have a corresponding match to the optical map. Other scaffolds are too small in size to be mapped using current optical map technology. However, during gap closure they were placed between contigs to fill gaps or hit to plasmids. The finishing strategy including gap closure was simplified using the optical map as an assembly model. The contigs were broken and inverted as indicated by the mapping and the gaps filled by PCR sequencing. Panel B: Comparison of the final assembly of the Xcaw genome (bottom) to the optical map (top) for the BamH1 digest.

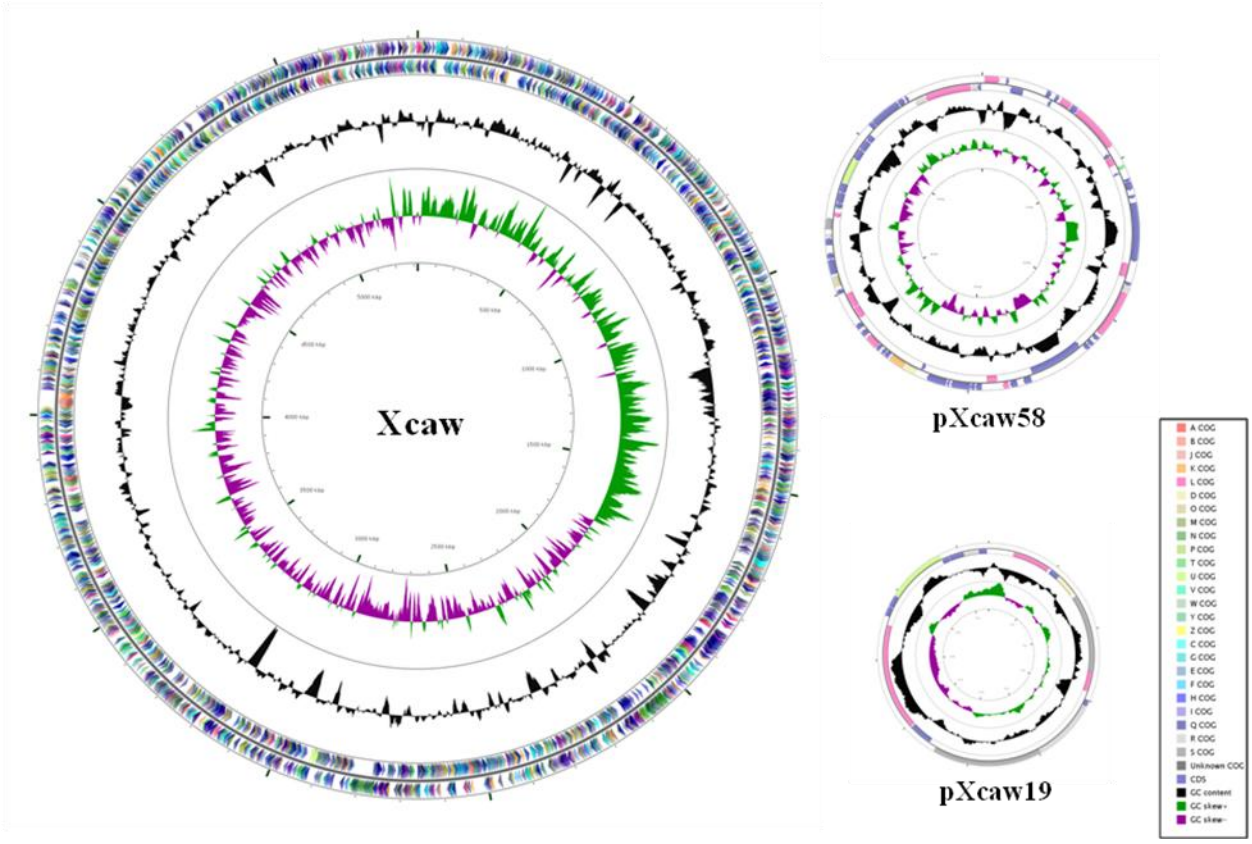


Figure 3-2. Circular representation of *X. citri* subsp. *citri* str. A^w 12879 genome and plasmids pXcaw19 & pXcaw58. Circles from outside to inside: first and second, predicted coding sequences of chromosome and plasmids on leading and lagging strands respectively (colors according to COGs); third, G+C content; forth, G+C skew; fifth, scale bar in kilobases.

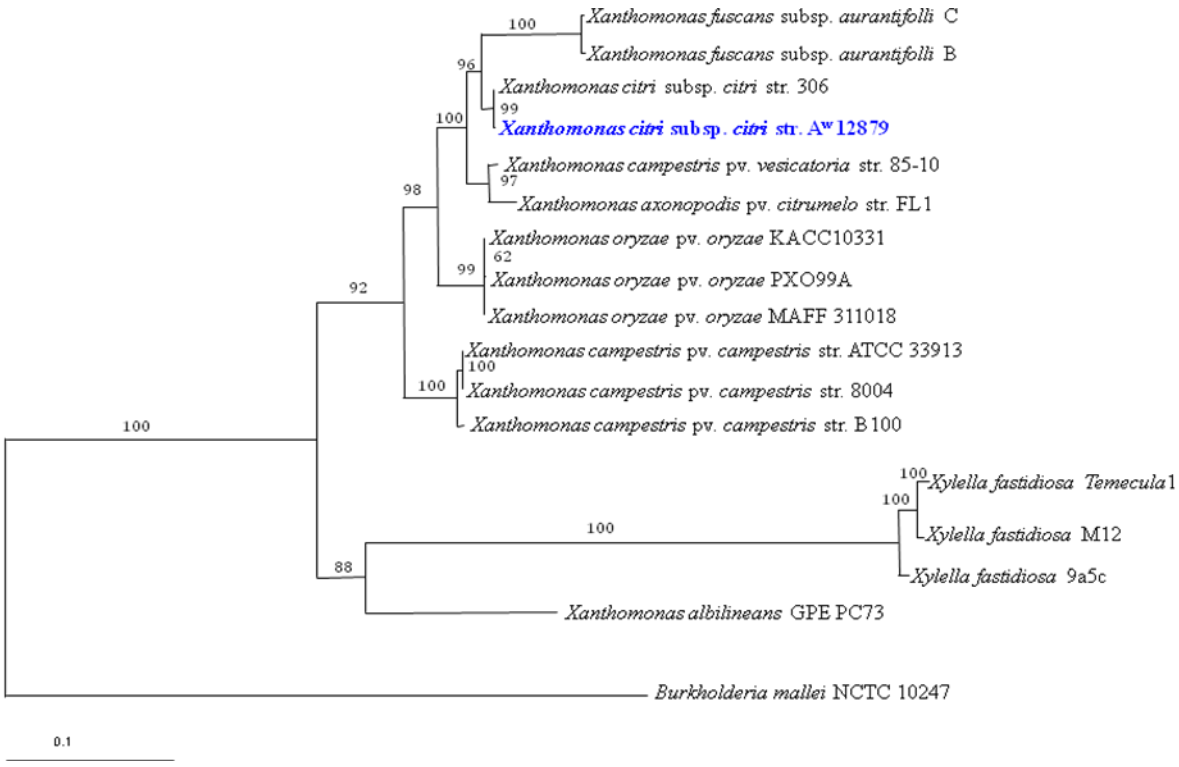


Figure 3-3. Maximum likelihood tree of the genome of *Xanthonomas citri* subsp. *citri* A^{w} 12879 showing the relationship to other fully sequenced Xathomonads (except XauB and XauC) and related species. The tree was constructed using concatenated protein sequences of nine housekeeping genes (*uvrD, secA, carA, recA, groEL, dnaK, atpD, gyrB* and *infB*) aligned using Clustal W. Phylogenic tree from concatenated sequences was constructed in PAUP (version 4.0) using the Maximum likelihood method. The sequence of *Burkholderia mallei* NCTC 10247 was used as out-group species. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Horizontal scale bar (0.1) at the bottom represents number of amino-acid substitutions per site.

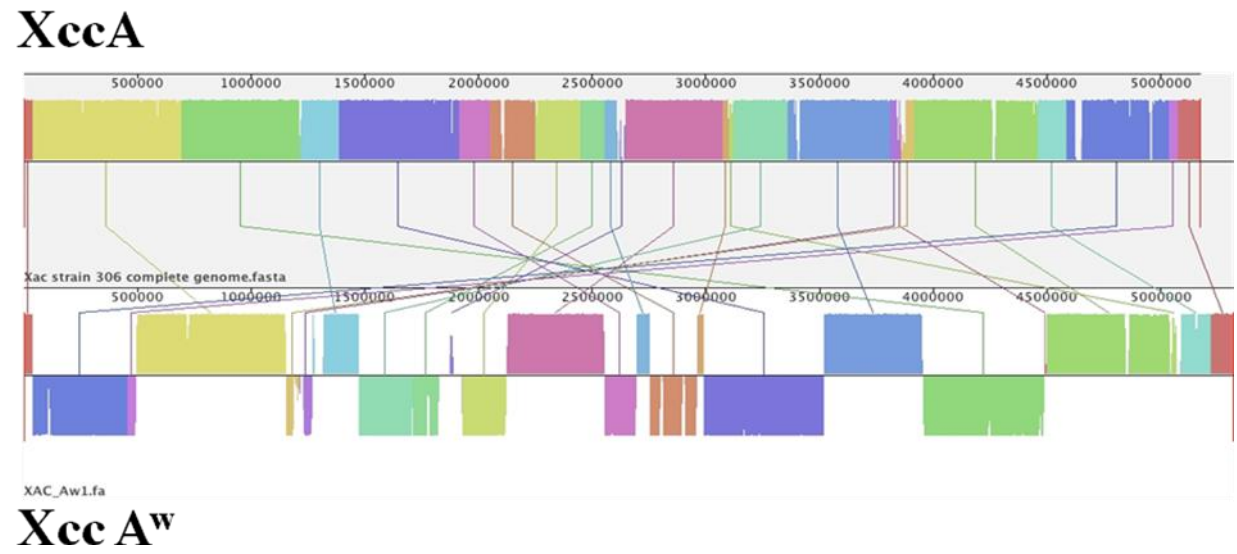


Figure 3-4. MAUVE alignment of the genome sequences of *X. citri* subsp. *citri* str. 306 and *X. citri* subsp. *citri* A^w 12879. Conserved and highly related regions are colored and low identity unique region are in white (colorless). The colored lines indicate translocations of the genome sections. Same colored blocks on opposite sides of the line indicate inversion.

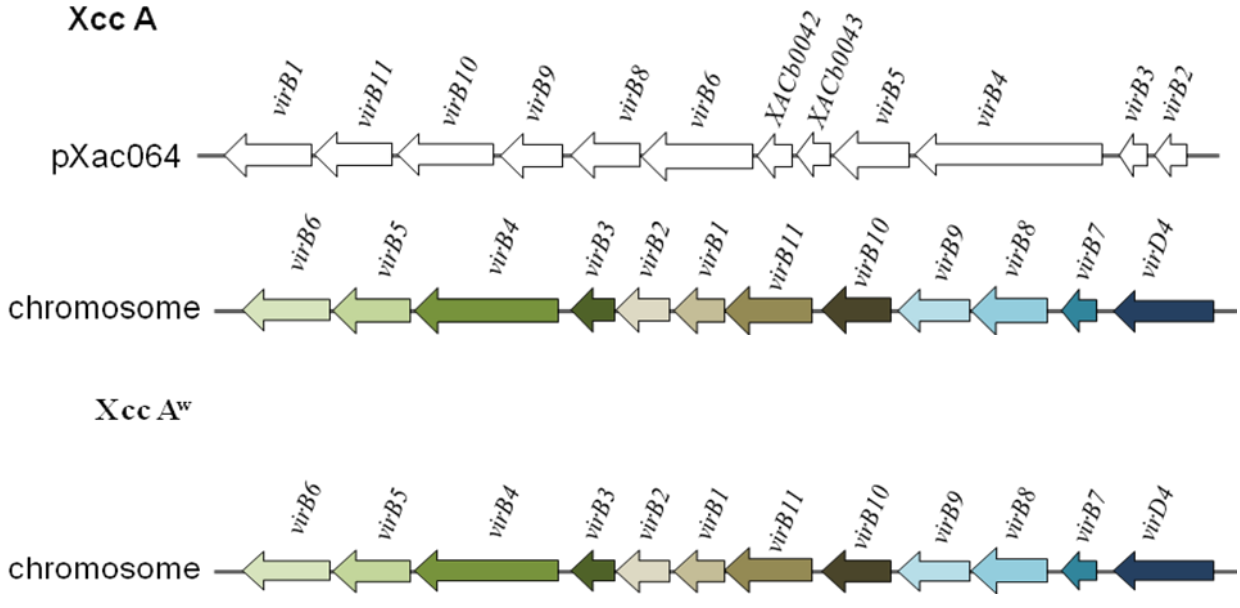


Figure 3-5. Comparison of the T4SS gene clusters of *X. citri* subsp. *citri* str. 306 (A), and *X. citri* subsp. *citri* str. A^w. Arrows indicate individual genes and homologues genes have same color. Aw does not contain the T4SS genes encoded by the plasmid Xac64 of XccA.

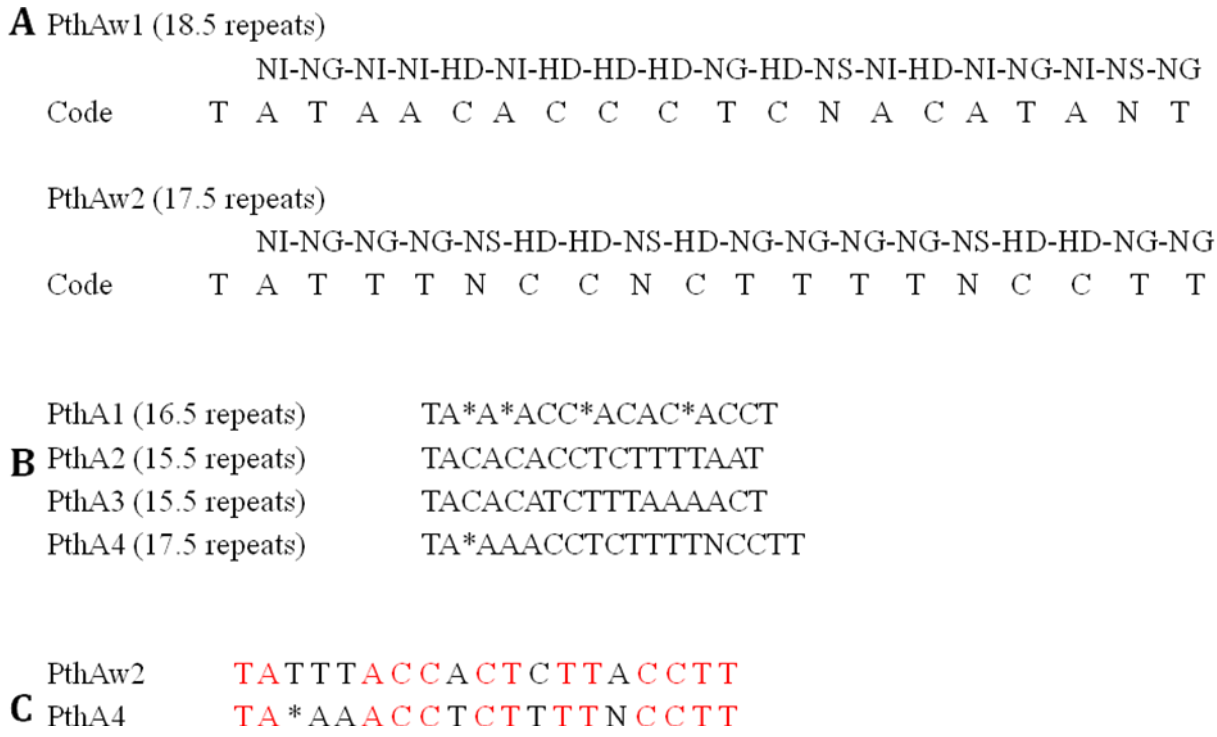


Figure 3-6. Prediction and comparison of the TAL effector codes encoded by *pthA* genes of *X. citri* subsp. *citri* str. 306, and *X. citri* subsp. *citri* str. A^{w}. Panel A: Prediction of TAL effector codes of PthAw1 and PthAw2. Panel B: The known TAL effector codes of PthA genes from XccA. Panel C: Comparison of the TAL effector codes of PthAw2 and PthA4, homologs in Xcaw and XccA respectively (Red residues show homology).

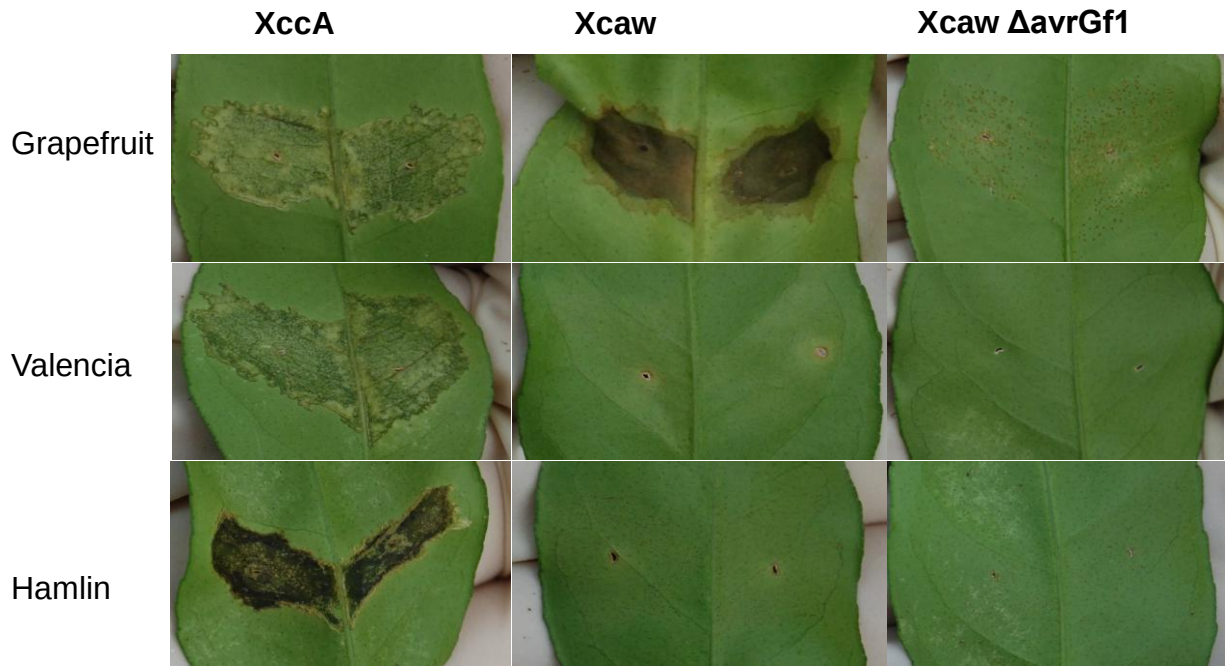


Figure 3-7. Inoculation by pressure infiltration of *X. citri* subsp. *citri* str. 306, *X. citri* subsp. *citri* str. A^w and *X. citri* subsp. *citri* str. A^w avrGf1 deletion mutant on young Grapefruit, Valencia and Hamlin leaves. The culture concentration of 108 was used for inoculation in plants were incubalted for 2 weeks. XccA infects all three citrus varities, Xcaw shows hypersencitive reaction only on Grapefruit. Xcaw avrGf1 deletion mutant shows reduced symptoms as compared to XccA on Grapefruit and no symptoms on Valencia and Hamlin.

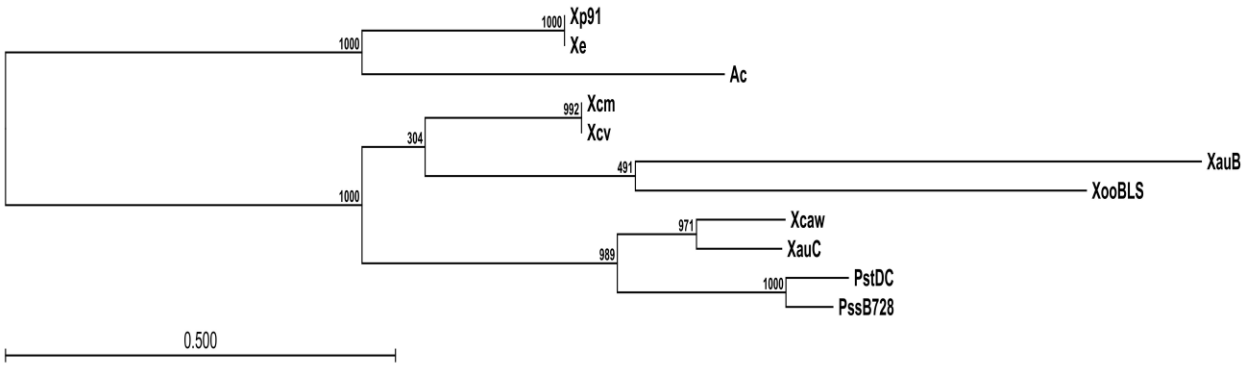


Figure 3-8. Neighbor joining tree of XopAF protein sequences. The tree was constructed using clustal aligned protein sequences of XopAF and its homologs. Bootstrap values are displayed at nodes. Xcaw, *X. citri* subsp. *citri* strain A^w 12879; XauB, XauC, *X. fuscans* subsp. *aurantifolii* strains B and C; XooBLS, *X. oryzae* pv. *oryzicola* BLS256; Xcv, *X. campestris* pv. *vesicatoria*; Xcm, *X. campestris* pv. *musacearum* NCPPB 4381; Xcv, *X. campestris* pv. *vasculorum* NCPPB 702; Xe, *X. euvesicatoria*; Xp91, *X. perforans* strain 91-118; Ac, *Acidovorax citrulli* AAC00-1; PstDC, *P. syringae* pv. *tomato* DC3000; PssB728, *P. syringae* pv. *syringae* B728. Horizontal scale bar (0.5) at the bottom represents number of amino-acid substitutions per site.

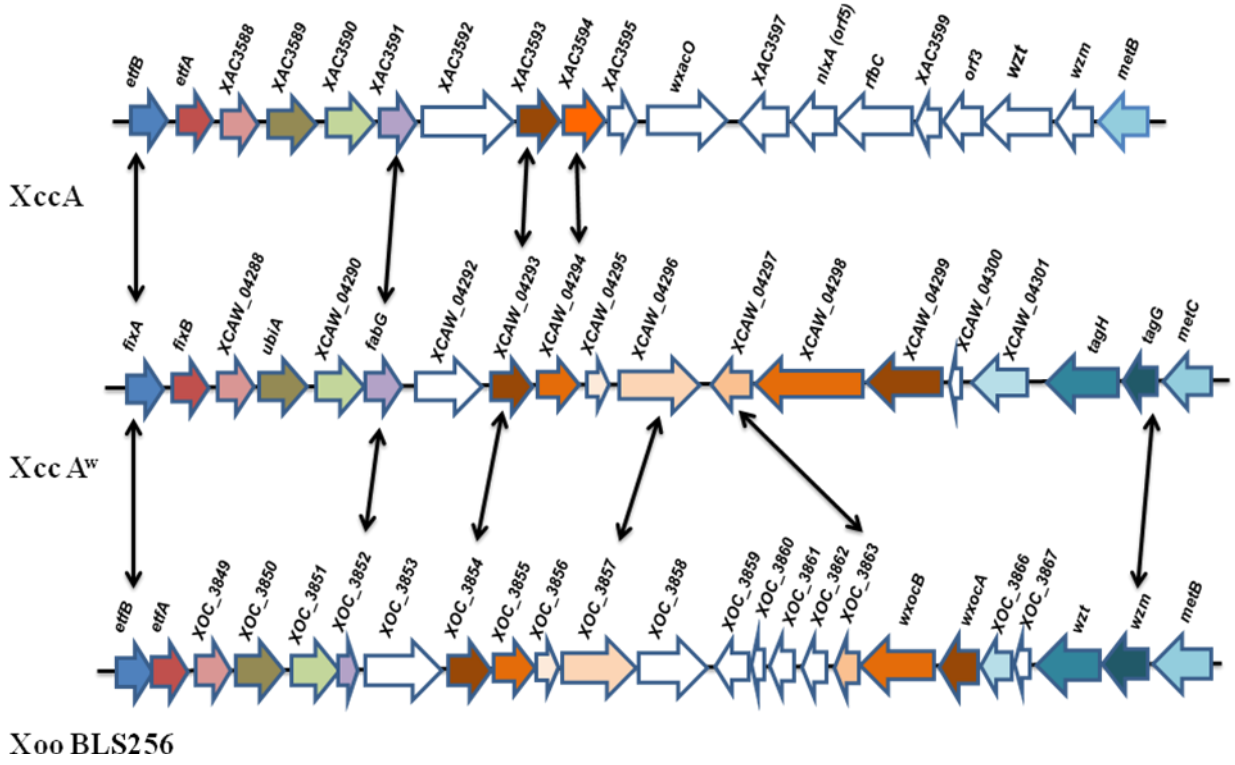


Figure 3-9. Comparison of the LPS gene clusters of *X. citri* subsp. *citri* str. 306, *X. citri* subsp. *citri* A^w 12879 and *X. oryzae* pv. *oryzicola* str. BLS256. Conserved and highly related genes (over 70% identity) are colored.

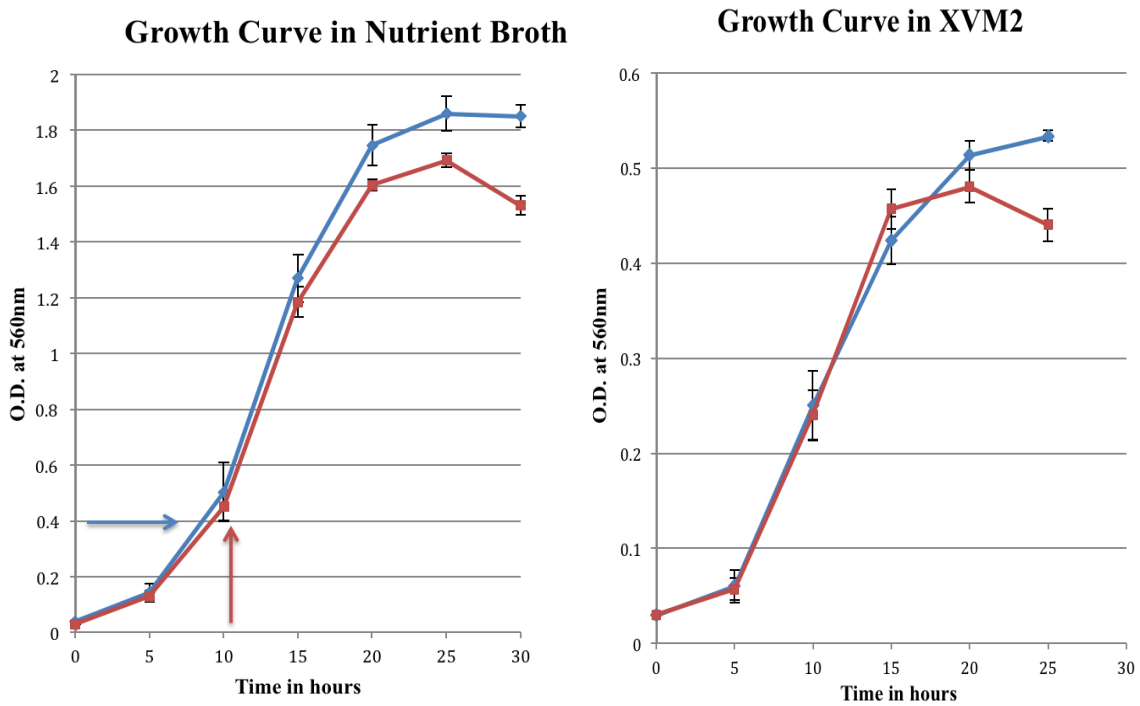


Figure 3-10. Growth of *X. citri* subsp. *citri* str. 306 (blue), and *X. citri* subsp. *citri* str. A^w 12879 (red) under NB and XVM2 conditions. Arrows indicate where the cells were harvested for RNA purification (O.D. 0.4 at 560 nm in each condition).

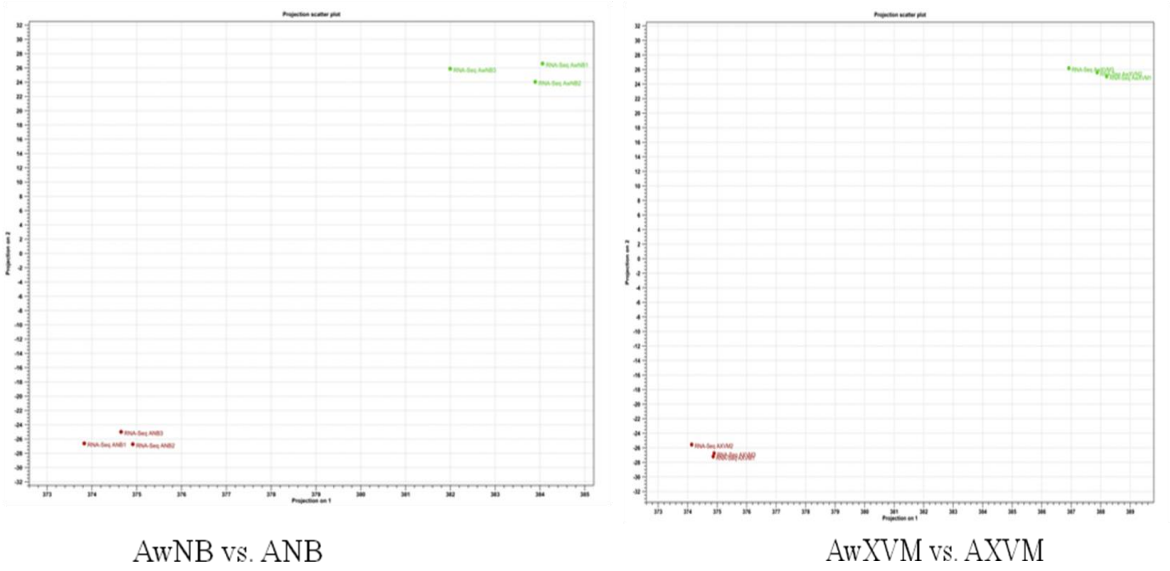


Figure 3-11. Principal component analysis of DEG of *X. citri* subsp. *citri* str. 306 (A), and *X. citri* subsp. *citri* str. A^w 12879 (Aw) under NB and XVM2 conditions. The genes expressed by Aw cluster separately from A in both the NB and XVM2 conditions indicating that orthologous genes are differentially expressed.

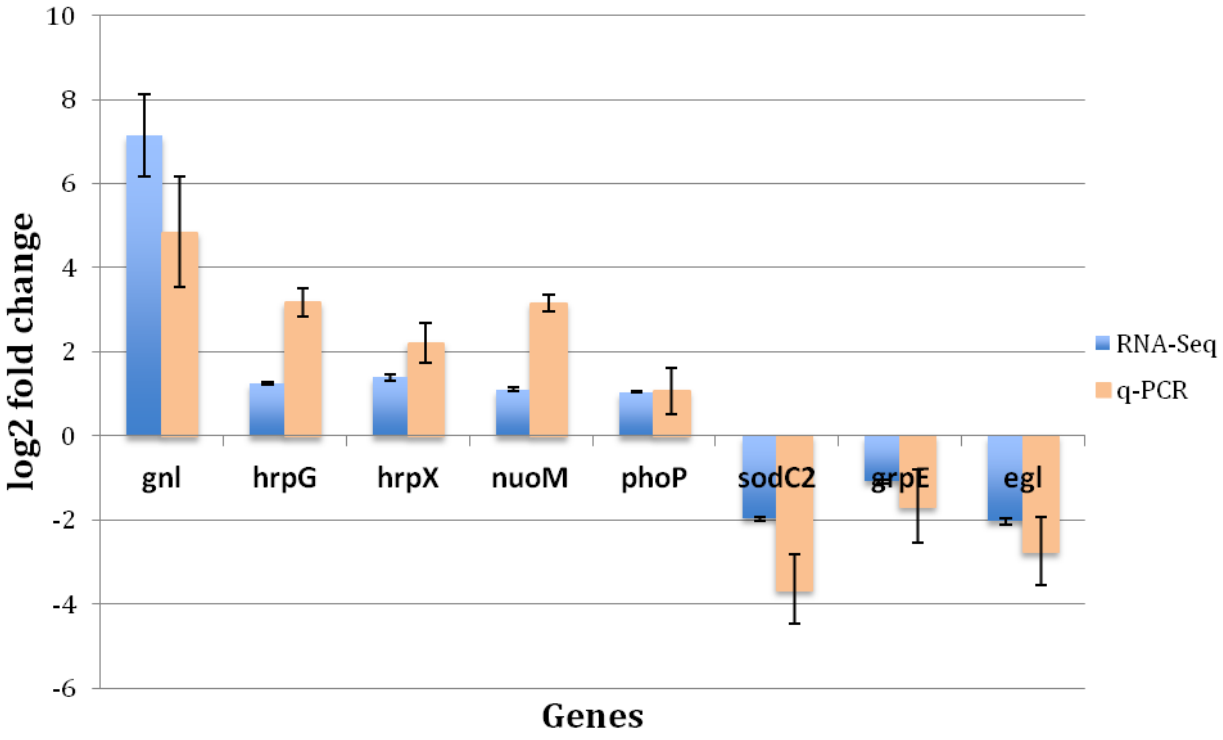


Figure 3-12. RNA-seq validation by qRT-PCR. Comparison of gene expression by quantitative reverse-transcription polymerase chain reaction (qRT-PCR) and RNA-seq. The log2-fold change of each gene was derived from comparison of either WNB vs ANB or WXVM2 vs AXVM2. The 16S rRNA gene was used as an endogenous control in qRT-PCR. Values of log2 fold change are means of three biological replicates. Error bars indicate standard deviation. Blue bars represent values from RNA-seq and yellow bars are values from qRT-PCR.

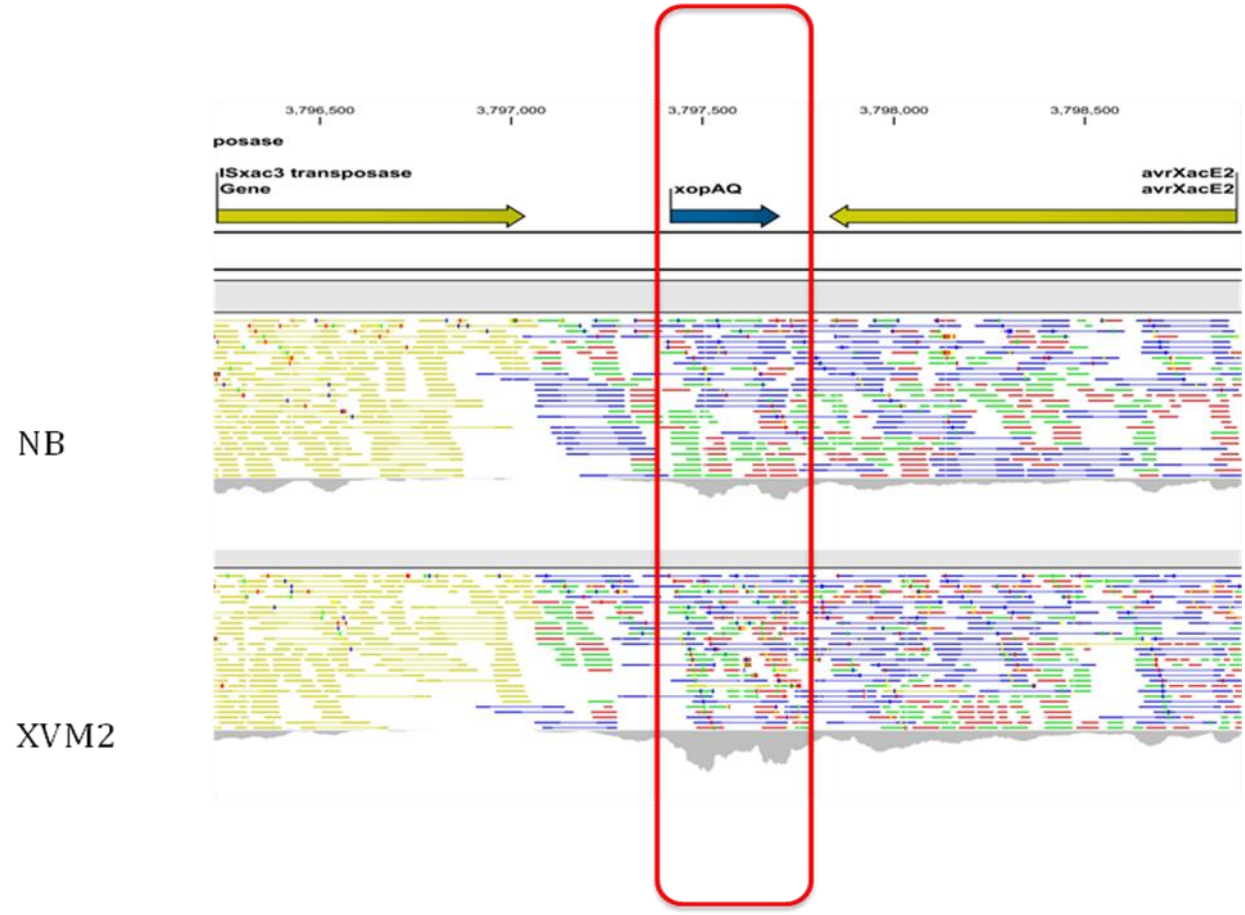


Figure 3-13. Identification of new genes by RNA-seq. Comparison of gene expression by aligning reads for XccA in NB and XVM2 medium to the genome. Differential expression was seen in intergenic region between ISxac3 transposase and avrXacE2 genes under different conditions (gray coverage scales below the read alignment). Blast analysis revealed presence of a putative effector protein encoding xopAQ gene. The log2-fold change of *xopAQ* from comparison of AXVM vs ANB is 1.81 (|fold change| = 3.51).

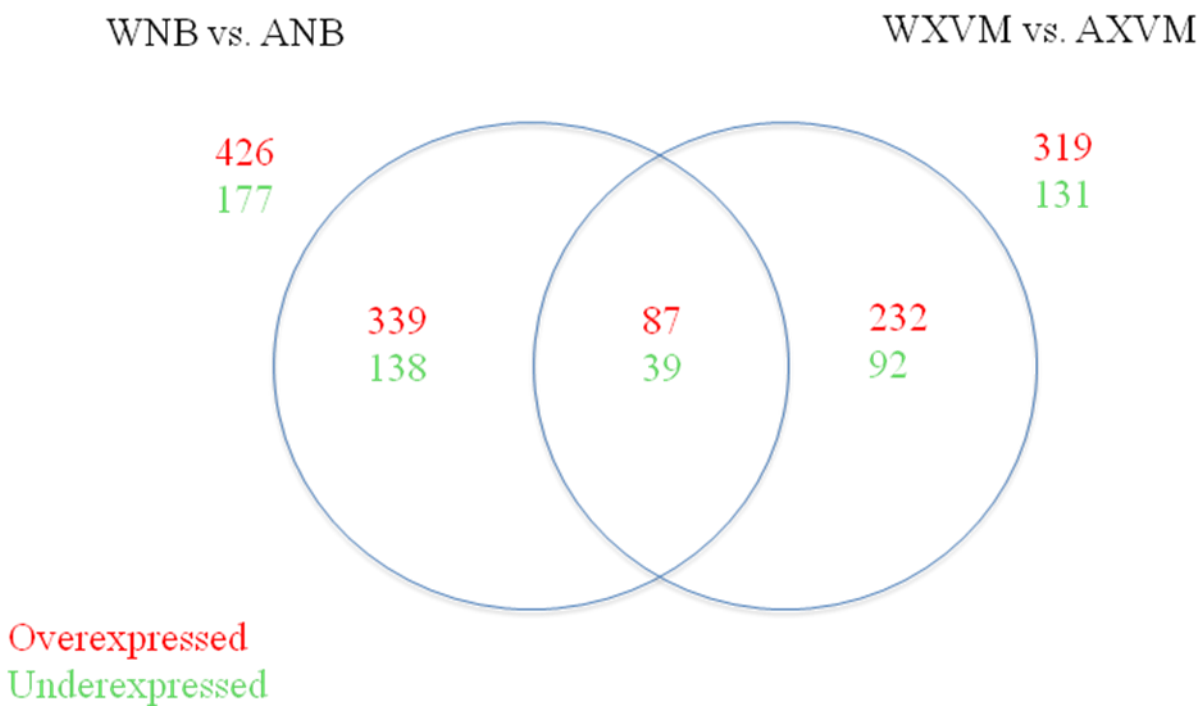


Figure 3-14. Number of differentially expressed genes when comparing expression of common genes in *X. citri* subsp. *citri* str. A^w 12879 against *X. citri* subsp. *citri* str. 306 in NB and XVM2 growth conditions. Gene expression of orthologous genes between Xcc Aw (W) and XccA (A) was compared when grown in Nutrient broth (NB, nutrient rich medium) and XVM2 (XVM, plant mimic medium).

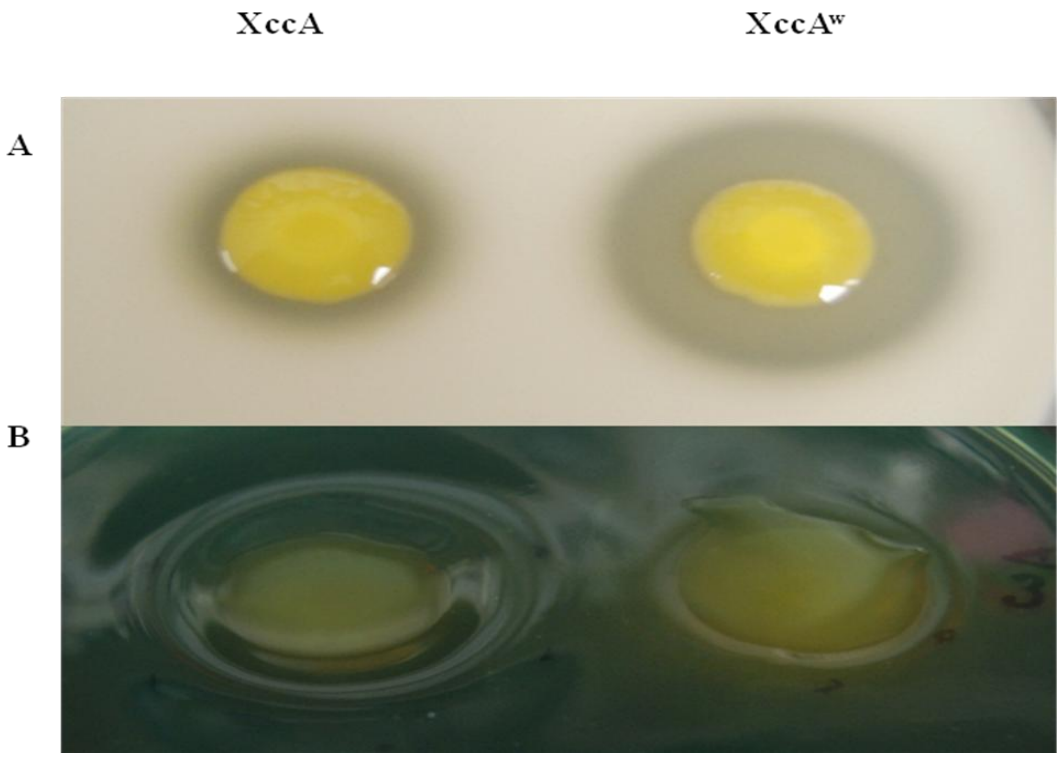


Figure 3-15. Protease and Pectate lyase activity of *X. citri* subsp. *citri* str. 306, and *X. citri* subsp. *citri* str. A^w 12879. A) Protease activity was tested by inoculating 1 µl culture on 10% milk agar plates at 28°C for 6 days. Zone of clearance was used as the measure of protease activity. B) Pectate lyase activity was tested by inoculating 1 µl culture on Hildebrand's agar medium at 28°C for 6 days. More pitting can be seen on medium at pH 8.5 for XccA strain as compared to Xcaw.

CHAPTER 4
SUMMARY AND CONCLUSION

Citrus canker caused by Xcc is one of the most serious diseases of citrus. XccA is distinguished into different variants primarily by host range. XccA has a wide host range and is most virulent, whereas Xcaw strain has host range restricted to Mexican lime and alemow. Xacm is another citrus pathogen causing citrus bacterial spot disease with limited host range and virulence. Both Xacm amd Xcaw are geographically restricted within the state of Florida. The goals of this study are to use comparative genomics and transcriptomics to understand the molecular mechanisms responsible for the difference in host range and virulence of these closely related *Xanthomonas* strains. It has been previously known that avirulence genes can affect host range of *Xanthomonas* and other plant pathogenic bacteria. Also various genes involved in virulence such as T1SS - T6SS, T3SS effectors, EPS, LPS, adhesins, flagellum and other have been previously identified by molecular as well as *in silico* studies. None of the studies however focused on the effect of gene expression on host-range and virulence of the phytopathogen. We hypothesized that gene expression along with gene content can contribute to virulence and host range of closely related strains.

In order to identify potential host range and virulence affecting factors we conducted a comprehensive comparative genomic study of an aggressive bacterial spot strain Xacm F1 to XccA. Illumina, 454 sequencing and optical mapping were used to obtain complete genome of Xacm strain F1 which is 4.9Mb chromosome with no plasmid. Phylogenetic studies indicated that Xacm is closely related to Xcv strain 85-10, which causes bacterial spot on tomato and pepper. Comparison of chromosome organization using MAUVE showed inversion (with translocation) and major deletions in

Xacm compared to XccA and Xcv. Comparison of the proteins of three *Xanthomonas* spp. showed differences in T3SS effectors, T4SS, LPS and others. In addition to *pthA*, effectors such as *xopE3*, *xopAI* and *hrpW* were absent in Xacm, which might be responsible for reduced virulence of this pathogen compared to XccA. We also identified unique effectors like *xopC2* and *xopW* in Xacm that may be restricted its host range. Xacm also lacks various toxin related genes, such as *syrE1*, *syrE2*, and RTX toxin family genes which are present in XccA. The absence of these genes may be associated with distinct virulence of XccA and Xacm.

We also sequenced the genome of Xcaw (5.3 Mb chromosome and two plasmids pXcaw19 and pXcaw58). Whole genome comparison of A^w to A strain, disclosed numerous genome rearrangements and insertion/deletion regions indicating genome plasticity. Comparative genomic analysis of Xcaw and XccA indicate that Xcaw strain specific effectors XopAG and XopAF might contribute to its limited host range compared to XccA. Also various changes in genes encoding LPS and T4SS for Xcaw were observed. RNA-seq was used to compare expression profile of Xcaw and XccA strains in nutrient rich (NB) and plant intercellular space mimicking (XVM2) conditions using Illumina sequencing. We found 5 avirulence/effector genes overexpressed in Xcaw compared to XccA. This might also contribute to its limited host range. The overexpression of genes involved in cell wall degradation, attachment, ROS scavenging, nutrient transportation in XccA might contribute to its expanding of host range. Our data also demonstrate that virulence genes including genes encoding TIIISS and its effectors are induced in the condition mimicking the plant intercellular environment.

Overall the comparison of the finished genomes of Xacm and Xcaw to XccA provides valuable insights into the emergence of new virulent strains with different host range and distinct virulences. It was observed that both the strong and weak citrus pathogens were capable of infecting Mexican lime effectively. This indicates that either Mexican lime is not equipped with various immunity realted genes or the pathogen can easily circumvent this immunity. Thus comparison of Mexican lime with other comparatively resistant varities of citrus should help uncover the plant genes important in fighting the citrus canker infection. Our transcriptome data also suggests that both gene content and gene expression contribute to difference in virulence and host range of different bacterial strains and this might be further true for plant genes especially those related to immunity. This study also lays foundation to further characterize the specific genes and the mechanism of difference in virulence and host range of stains of *X. citri* subsp*. citri* and other bacterial pathogens.

APPENDIX
MEDIA COMPOSITION

NUTRIENT AGAR (NA)

Beef Extract	3.0 g
Peptone	5.0 g
Agar	15.0 g
DDW	1000 ml

NUTRIENT BROTH (NB)

Beef Extract	3.0 g
Peptone	5.0 g
DDW	1000 ml

XVM2

Sodium chloride	1.16 g
Ammonium sulphate	1.32 g
Magnesium sulphate	0.6 g
Calcium chloride	0.147 g
Potassium dihydrogen phosphate	160 µl of 1 M stock
DiPotassium hydrogen phosphate	320 µl of 1 M stock
Ferrous sulphate	40 µl of 250 mM stock
DDW	980 ml

Adjust pH to 6.7, autoclave and cool

Fructose	10 ml of 1 M stock
Sucrose	10 ml of 1 M stock
Casaminoacid	1 ml of 30% stock

HILDEBRAND'S MEDIUM (3 different pH media)

DDW	1000 ml

Heat to near boiling

Bromothymol blue	1.5% alcoholic solution
Calcium chloride	6 ml of 10% stock
Sodium polypectate	22 g
Agar	100 ml of 4% solution

Adjust pH for two to 4.5-4.7 and 6.9-7.1 with 1N HCl solution and autoclave
Autoclave the third and adjust pH 8.3-8.5 with 1N NaOH

SKIM MILK AGAR PLATE

Nutrient Agar 500 ml. Autoclave Pour basal plate (thin layer) and cool.

2X Nutrient Agar 250 ml
Skim milk Dissolve 50 gm skim milk powder completely in 250 ml warm DDW
Autoclave and mix st. skim milk with 2X NA to get 10% Skim milk agar and pour top layer on basal plates.

LIST OF REFERENCES

Achor DS, Browning HW, and Albrigo LG. 1996. Anatomical and histological modification in citrus leaves caused by larval feeding of citrus leaf-miner (*Phyllocnistis citrella* Staint). Page 69 in: *Proc Int Conf Citrus Leafminer*, Orlando, Florida, April 23-25.

Al-Saadi A. 2005. Phenotypic characterization and sequence analysis of pthA homologs from five pathogenic variant groups of *Xanthomonas citri*. University of Florida, Gainesville, Florida.

Al-Saadi A, Reddy JD, Duan YP, Brunings AM, Yuan Q, and Gabriel DW. 2007. All five host-range variants of *Xanthomonas citri* carry one *pthA* homolog with 17.5 repeats that determines pathogenicity on citrus, but none determine host-range variation. *Mol Plant Microbe Interact* **20:**934-943.

Alegria M, Souza D, Andrade M, Docena C, Khater L, Ramos C, da Silva A, Farah C**.** 2005. Identification of new protein-protein interactions involving the products of the chromosome and plasmid-encoded type IV secretion loci of the phytopathogen *Xanthomonas axonopodis* pv. *citri*. *J Bacteriol* **187:**2315-2325.

Alfano JR, Collmer A. 1997. The type III (Hrp) secretion pathway of plant pathogenic bacteria: trafficking harpins, Avr proteins, and death. *J. Bacteriol*. **179**:5655-5662.

Alvarez AM, Benedict AA, Mizumoto CY, Pollard LW, Civerolo EL. 1991. Analysis of *Xanthomonas campestris* pv. *citri* and *X. c. citrumelo* with monoclonal antibodies. *Phytopathology* **81:**9.

Andrade M, Alegria M, Guzzo C, Docena C, Rosa M, Ramos C, Farah C. 2006. The HD-GYP domain of RpfG mediates a direct linkage between the Rpf quorum-sensing pathway and a subset of diguanylate cyclase proteins in the phytopathogen *Xanthomonas axonopodis* pv *citri*. *Mol Microbiol* **62:**537-551.

Arnold DL, Jackson RW. 2011. Bacterial genomes: evolution of pathogenicity. *Current opinion in Plant Biology* **14**:385-391.

Astua-Monge G, Minsavage GV, Stall RE, Davis MJ, Bonas U, Jones JB. 2000. Resistance of tomato and pepper to T3 strains of *Xanthomonas campestris* pv. *vesicatoria* is specified by a plant-inducible avirulence gene. *Mol Plant Microbe Interact* **13**:911-621.

Astua-Monge G, Feritas-Astua J, Bacocina G, Roncoletta J, Carvalho SA, Machado MA. 2005. Expression profiling of virulence and pathogenecity related genes of *Xanthomonas axonopodis* pv. *citri*. *J Bacteriol* **187**:1201-1205.

Aury JM, Cruaud C, Barbe V, Rogier O, Mangenot S, Samson G, Poulain J, Anthouard, Scarpelli VC, Artiguenave F, Wincker P. 2008. High quality draft sequences for

prokaryotic genomes using a mix of new sequencing technologies. *BMC Genomics* **9:**603.

Backert S, Meyer TF. 2006. Type IV secretion systems and their effectors in bacterial pathogenesis. *Curr Opin in Microbiol* **9**:207-217.

Barber CE, Tang JL, Feng JX, Pan MQ, Wilson TJ, Slater H, Dow JM, Williams P, Daniels MJ. 1997. A novel regulatory system required for pathogenicity of *Xanthomonas campestris* is mediated by a small diffusible signal molecule. *Mol*. *Microbiol*. **24**:555-566.

Barrett LG, Kniskern JM, Bodenhausen N, Zhang W, Bergelson J. 2009. Continua of specificity and virulence in plant host-pathogen interactions: causes and consequenses. *New Phytologist* **183**:513-529.

Bartetzko V, Sonnewald S, Vogel F, Hartner K, Stadler R, Hammes UZ, Börnke F. 2009. The *Xanthomonas campestris* pv. *vesicatoria* type III effector protein XopJ inhibits protein secretion: evidence for interference with cell wall-associated defense responses. *Mol Plant Microbe Interact* **22:**655-664.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Royal Stat Soc* **67**:289-300.

Bentley DR. 2006. Whole-genome re-sequencing. *Curr Opin Genet Dev* **16:**545-552.

Boch J, Bonas U. 2010. *Xanthomonas* AvrBs3 type family-type III effectors: discovery and function. *Phytopathology* **48**:419-436.

Bogdanove AJ, Koebnik R, Lu H, Furutani A, Angiuoli SV, Patil PB, VanSluys MA, Ryan RP, Meyer DF, Han SW, et al. 2011. Two new complete genome sequences offer insight into host and tissue specificty of plant pathogenic *Xanthomonas* spp. *J Bacteriol* **193:**5450-5464.

Boller T, He SY, 2009. Innate immunity in plants: an arms race between pattern recognition receptors in plants and effectors in microbial pathogens. *Science* **324**:742-744.

Boyer F, Fichant G, Berthod J, Vandenbrouck Y, Attree I. 2009. Dissecting the bacterial type VI secretion system by a genome wide in silico analysis: what can be learned from available microbial genomic resources? *BMC Genomics* **10:**104.

Brunings AM, Gabriel DW. 2003. *Xanthomonas citri*: breaking the surface. *Molecular Plant Pathology* **4**:141-157.

Buttner D, Bonas U. 2002. Getting across--bacterial type III effector proteins on their way to the plant cell. *EMBO (Eur. Mol. Biol. Organ.) J.* **21**:5313-5322.

Büttner D, Bonas U. 2003. Common infection strategies of plant and animal pathogenic bacteria. *Curr Opin Plant Biol* **6:**312-319.

Büttner D, Bonas U. 2010. Regulation and secretion of *Xanthomonas* virulence factors. *FEMS Microbiol Rev* **34:**107-133.

Canteros BI. 2002. Control of citrus canker caused by *Xanthomonas axonopodis* pv. *citri* susceptible and resistant to copper. *Phytopathology* **92**:S116.

Cascales E. 2008. The type VI secretion toolkit. *EMBO (Eur. Mol. Biol. Organ.) Rep*. **9**:735-741.

Chan JW, Goodwin PH. 1999. The molecular genetics of virulence of *Xanthomonas campestris*. *Biotechnol Adv* **17**(6):489-508.

Chatterjee S, Sonti RV. 2002. *rpfF* mutants of *Xanthomonas oryzae* pv. *oryzae* are deficient for virulence and growth under low iron conditions. *Mol. Plant-Microbe Interact*. **15**:463-471.

Chisholm ST, Coaker G, Day B, Staskawicz BJ. 2006. Host-microbe interactions: shaping the evolution of the plant immune response. *Cell* **124**:803-814.

Christie PJ, Atmakuri K, Krishnamoorthy V, Jakubowski S, Cascales E. 2005. Biogenesis, architecture, and function of bacterial type IV secretion systems. *Annu Rev Microbiol* **59:**451-485.

Chou FL, Chou HC, Lin YS, Yang BY, Lin NT, Weng SF, Tseng YH. 1997. The *Xanthomonas campestris gumD* gene required for synthesis of xanthan gum is involved in normal pigmentation and virulence in causing black rot. *Biochem. Biophys. Res. Commun.* **233**:265-269.

Civerolo EL. 1984. Bacterial canker disease of citrus. *J Rio Grande Val Hort Soc* **37**:127-145.

Clarke CR, Cai R, Studholme DJ, Guttman DS, Vinatzer BA. 2010. *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* **23:**198-210.

Cubero J, Graham JH. 2002. Genetic relationship among worldwide strains of *Xanthomonas* causing canker in citrus species and design of new primers for their identification by PCR. *Appl Environ Microbiol* **68:**1257-1264.

Cubero J, Graham JH. 2004. The leucine-responsive regulatory protein (lrp) gene for characterization of the relationship among *Xanthomonas* species. *Int J Syst Evol Microbiol* **54:**429-437.

da Silva AC, Ferro JA, Reinach FC, Farah CS, Furlan LR, Quaggio RB, Monteiro-Vitorello CB, Van Sluys MA, Almeida NF, Alves LM, et al. 2002. Comparison of the genomes of two *Xanthomonas* pathogens with differing host specificities. *Nature* **417**(6887):459-463.

da Silva FG, Shen Y, Dardick C, Burdman S, Yadav RC, de Leon AL, Ronald PC. 2004. Bacterial genes involved in type I secretion and sulfation are required to elicit the rice Xa21-mediated innate immune response. *Mol. Plant-Microbe Interact.* **17**:593-601.

Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One* **5**(6): e11147.

Das AK. 2003. Citrus canker - A review. *J Appl Hort* **5**(1):52-60.

Daubin V, Ochman H. 2004. Bacterial genomes as new gene homes: the genealogy of ORFans in E. coli. *Genome Res* **14:**1036-1042.

del Campo R, Russi P, Maa P, Mara H, Peyrou M, de León IP, Gaggero C. 2009. *Xanthomonas axonopodis* pv*. citri* enters the VBNC state after copper treatment and retains its virulence. *FEMS Microbiol Lett* **298**:143-8.

Dohm JC, Lottaz C, Borodina T, Himmelbauer H. 2008. Substantial biases in ultra-short read data sets from high-throughput DNA sequencing. *Nucleic Acids Res* **36:**e105.

Domingues MN, de Souza TA, Cernadas RA, de Oliveira MLP, Docena C, Farah CS, Benedetti CE. 2010. The *Xanthomonas citri* effector protein PthA interacts with citrus proteins involved in nuclear transport, protein folding and ubiquitination associated with DNA repair. *Mol Plant Path* **11:** 663-675.

Dow JM, Clarke BR, Milligan DE, Tang JL, Daniels MJ. 1990. Extracellular proteases from *Xanthomonas campestris* pv. *campestris*, the black rot pathogen. *Appl Environ Microbiol* **56:**2994-2998.

Dow J, Fouhy Y, Lucey J, Ryan R. 2006. The HD-GYP domain, cyclic di-GMP signaling, and bacterial virulence to plants. *Mol. Plant-Microbe Interact*. **19**:1378-1384.

Dow M, Newman MA, Roepenack EV. 2000. The induction and modulation of plant defence responses by bacterial lipopolysaccharides. *Annu Rev Phytopathol* **38**:241-261.

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

Egel DS, Graham JH, Stall RE. 1991. Genomic Relatedness of *Xanthomonas campestris* Strains Causing Diseases of Citrus. *Appl Environ Microbiol* **57:**2724-2730.

Etchegaray A, Silva-Stenico ME, Moon DH, Tsai SM. 2004. In silico analysis of nonribosomal peptide synthetases of *Xanthomonas axonopodis* pv. *citri*: identification of putative siderophore and lipopeptide biosynthetic genes. *Microbiol Res* **159:**425-437.

Fenselau S, Bonas U. 1995. Sequence and expression analysis of the *hrpB* pathogenicity operon of *Xanthomonas campestris* pv. *vesicatoria* which encodes eight proteins with similarity to components of the Hrp, Ysc, Spa, and Fli secretion systems. *Mol Plant Microbe Interact* **8:**845-854.

Filiatrault MJ, Stodghill PV, Bronstein PA, Moll S, Lindeberg M, Grills G, Schweitzer P, Wang W, Schroth GP, Luo S, et al. 2010. Transcriptome analysis of *Pseudomonas syringae* identifies new genes, noncoding RNAs and antisense activity. *J Bacteriol* **192**: 2359-2372.

Florida Citrus Mutual, Inc. 2012. www.flcitrusmutual.com/citrus-101/citrusstatistics.aspx last accessed on September 15, 2012.

Fu XZ, Gong XQ, Zhang YX, Wang Y, Liu JH. 2012. Different transcriptional response to *Xanthomonas citri subsp. citri* between Kumquat and Sweet Orange with contrasting canker tolerance. *Plos ONE* **7**(7):e41790.

Furutani A, Takaoka M, Sanada H, Noguchi Y, Oku T, Tsuno K, Ochiai H, Tsuge S. 2009. Identification of novel type III secretion effectors in *Xanthomonas oryzae* pv. *oryzae*. *Mol Plant Microbe Interact* **22:**96-106.

Gabriel DW, Hunter JE, Kingsley MT, Miller JW, Lazo GR. 1988. Clonal population structure of *Xanthomonas campestris* and genetic diversity among citrus canker strains. *Mol Plant Microbe Interact* **1**(2):59-65.

Gabriel DW, Kingsley MT, Hunter JE, Gottwald T. 1989. Reinstatement of *Xanthomonas citri* (ex Hasse) and *X. phaseoli* (ex Smith) to species and reclassification of all *X. campestris* pv. *citri* strains. *International Journal of Systematic Bacteriology* **39:**14-22.

Galperin MY. 2004. Bacterial signal transduction network in a genomic perspective. *Environ Microbiol* **6**(6): 552-567.

Gao F, Zhang CT. 2006. GC-Profile: a web-based tool for visualizing and analyzing the variation of GC content in genomic sequences. *Nucleic Acids Res* **34:**W686-691.

Garavaglia BS, Thomas L, Gottig N, Zimaro T, Garofalo CG, Gehring C, Ottado J. 2010a. Shedding light on the role of photosynthesis in pathogen colonization and host defense. *Commun Integr Biol* **3:**382-384.

Garavaglia BS, Thomas L, Zimaro T, Gottig N, Daurelio LD, Ndimba B, Orellano EG, Ottado J, Gehring C. 2010b. A plant natriuretic peptide-like molecule of the pathogen *Xanthomonas axonopodis* pv. *citri* causes rapid changes in the proteome of its citrus host. *BMC Plant Biol* **10:**51.

Gerlach RG, Hensel M. 2007. Protein secretion systems and adhesins: The molecular armory of Gram-negative pathogens. *Int. J. Medical Microbiol*. **297**:401-415.

Genin S. 2010. Molecular traits controlling host range and adaptation to plants in *Ralstonia solanacearum*. *New Phytologist* **187**:920-928.

Gent DH, Al-Saadi A, Gabriel DW, Louws FJ, Ishimaru CA, Schwartz HF. 2005. Pathogenic and genetic relatedness among *Xanthomonas axonopodis* pv. *allii* and other pathovars of *X. axonopodis*. *Phytopathology* **95:**918-925.

Gottig N, Garavaglia BS, Garofalo CG, Daurelio LD, Valentine A, Gehring C, Orellano EG, Ottado J. 2008. *Xanthomonas axonopodis* pv. *citri* uses a plant natriuretic peptide-like protein to modify host homeostasis. *Proc Natl Acad Sci USA* **105:**18631-18636.

Gottig N, Garavaglia BS, Garofalo CG, Orellano EG, Ottado J. 2009. A filamentous hemagglutinin-like protein of *Xanthomonas axonopodis* pv. *citri*, the phytopathogen responsible for citrus canker, is involved in bacterial virulence. *PLoS One* **4:**e4358.

Gottwald TR, Alvarez AM, Hartung JS, Benedict AA. 1991. Diversity of *Xanthomonas campestris* pv. *citrumelo* strains associated with epidemics of citrus bacterial spot in Florida citrus nurseries: Correlation of detached leaf, monoclonal antibody, and restriction fragment length polymorphism assays. *Phytopathology* **81**(7):749-753.

Gottwald TR, Civerolo EL, Garnsey SM, Brlansky RH, Graham JH, Gabriel DW. 1988. Dynamics and spatial distribution of *Xanthomonas campestris* pv. *citri* group E strains in simulated nursery and new grove situations. *Plant Diease* **72**(9):781-787.

Gottwald TR, Graham JH. 1990. Spatial pattern analysis of epidemics of citrus bacterial spot in Florida nurseries. *Phytopathology* **80**(2):181-190.

Gottwald TR, Graham JH, Civerolo EL, Barrett HC, Hearn CJ. 1993. Differential host range reaction of citrus and citrus relatives to citrus canker and citrus bacterial spot determined by leaf mesophyll susceptibility. *Plant Disease* **77**(10):1004-1009.

Gottwald TR, Hughes G, Graham JH, Sun X, Riley T. 2001. The citrus canker epidemic in Florida: the scientific bases of regulatory eradication policy for invasive species. *Phytopathology* **91**:30-34.

Gottwald TR, Riley T. 2005. The effect of hurricanes and tropical storms on long distance spread of citrus canker. *Phytopathology* **95**:6S36.

Gottwald TR, Graham JH, Schubert TS. 2002. Citrus Canker: The pathogen and its impact. *Plant Health Progress* doi:10.1094/PHP-2002-0812-01-RV.

Graham JH, Gottwald TR. 1991. Research perspectives on eradication of citrus bacterial diseases in Florida. *Plant Disease* **75**:1193-1200.

Graham JH, Gottwald TR. 1990. Variation in aggressiveness of *Xanthomonas campestris* pv. *citrumelo* associated with citrus bacterial spot in Florida citrus nurseries. *Phytopathology* **80**(2):190-196.

Graham JH, Gottwald TR, Fardelmann D. 1990. Cultivar-specific interactions for strains of *Xanthomonas campestris* from Florida that cause citrus canker and citrus bacterial spot. *Plant Disease* **74:**753-756.

Grant JR, Stothard P. 2008. The CGView Server: a comparative genomics tool for circular genomes. *Nucleic Acids Res* **36:**181-184.

Grant SR, Fisher EJ, Chang JH, Mole BM, Dangl JL. 2006. Subterfuge and manipulation: type III effector proteins of phytopathogenic bacteria. *Annu Rev Microbiol* **60:**425-449.

Guidot A, Prior P, Schoenfeld J, Carrère S, Genin S, Boucher C. 2007. Genomic structure and phylogeny of the plant pathogen *Ralstonia solanacearum* inferred from gene distribution analysis. *J Bacteriol* **189:**377-387.

Guo Y, Figueiredo F, Jones J, Wang N. 2011. HrpG and HrpX play global roles in coordinating different virulence traits of *Xanthomonas axonopodis* pv. *citri*. *Mol Plant Microbe Interact* **24:**649-661.

Guo Y, Sagaram U, Kim JS, Wang N. 2010. Requirement of the *galU* gene for polysaccharide production by and pathogenicity and growth in planta of *Xanthomonas citri* subsp. *citri*. *Appl. Environ. Microbiol*. **76**: 2234-2242.

Guo Y, Zhang Y, Li JL, Wang N. 2012. Diffusible signal factor-mediated qorum sensing plays a central role in coordinating gene expression of *Xanthomonas citri* subsp. *citri*. *Mol Plant Microbe Interact* **25**: 165-179.

Gürlebeck D, Thieme F, Bonas U. 2006. Type III effector proteins from the plant pathogen *Xanthomonas* and their role in the interaction with the host plant. *J Plant Physiol* **163:**233-255.

Hajri A, Brin C, Hunault G, Lardeux F, Lemaire C, Manceau C, Boureau T, Poussier S. 2009. A "repertoire for repertoire" hypothesis: repertoires of type three effectors are candidate determinants of host specificity in *Xanthomonas*. *PLoS One* **4:**e6632.

Han SW, Lee SW, Ronald PC. 2011. Secretion, modification, and regulation of Ax21. *Curr Opin Microbiol* **14:**62-67.

Hartung JS, Civerolo ES. 1989. Restriction fragment length polymorphisms distinguish *Xanthomonas campestris* strains isolated from Florida citrus nurseries from *X. c.* pv. *citri*. *Mol Plant Pathol* **79**(7):793-799.

Hauben L, Vauterin L, Moore ER, Hoste B, Swings J. 1999. Genomic diversity of the genus *Stenotrophomonas*. *Int J Syst Bacteriol* **49**(4):1749-1760.

He Y, Ng A, Xu M, Lin K, Wang L, Dong Y, Zhang L. 2007. *Xanthomonas campestris* cell-cell communication involves a putative nucleotide receptor protein Clp and a hierarchical signalling network. *Mol Microbiol* **64**:281-292.

He Y, Xu M, Lin K, Ng Y, Wen C, Wang L, Liu Z, Zhang H, Dong Y, Dow J, Zhang L. 2006. Genome scale analysis of diffusible signal factor regulon in *Xanthomonas campestris* pv. *campestris*: identification of novel cell-cell communication-dependent genes and functions*. Mol Microbiol* **59**:610-622.

Hildebrand DC. 1971. Pectate and pectin gels for differentiation of *Pseudomonas* sp. and other bacterial plant pathogens. *Phytopathology* **61**:1430-1436.

Hirano SS, Upper CD. 2000. Bacteria in the leaf ecosystem with emphasis on *Pseudomonas syringae*-a pathogen, ice nucleus, and epiphyte. *Microbiol Mol Biol Rev* **64**:624-653.

Hueck CJ. 1998. Type III protein secretion systems in bacterial pathogens of animals and plants. *Microbiol Mol Biol Rev* **62**:379-433.

Huse SM, Huber JA, Morrison HG, Sogin ML, Welch DM. 2007. Accuracy and quality of massively parallel DNA pyrosequencing. *Genome Biol* **8**(7):R143.

Jalan N, Aritua V, Kumar D, Yu F, Jones JB, Graham JH, Setubal JC, Wang N. 2011. Comparative genomic analysis of *Xanthomonas axonopodis* pv. *citrumelo* F1, which causes citrus bacterial spot disease and related strains provides insights into virulence and host specificity. *J Bacteriol* **193**:6342-6357.

Jiang Y. 2007. The rice XA21-binding protein 25 is an ankyrin repeat-containing protein and required for XA21-mediated disease resistance. University of Florida, Gainesville, Florida.

Jiang W, Jiang BL, Xu RQ, Huang JD, Wei HY, Jiang GF, Cen WJ, Liu J, Ge YY, Li GH, et al. 2009. Identification of six type III effector genes with the PIP box in *Xanthomonas campestris* pv. *campestris* and five of them contribute individually to full pathogenicity. *Mol Plant Microbe Interact* **22:**1401-1411.

Jones JDG, Dangl JL. 2006. The plant immune system. *Nature* **444**:323-329.

Jones JB, Stall RE, Bouzar H. 1998. Diversity among xanthomonads pathogenic on pepper and tomato. *Annu Rev Phytopathol* **36:**41-58.

Josenhans C, Suerbaum S. 2002. The role of motility as a virulence factor in bacteria. *Int J Med Microbiol* **291**:605-614.

Kay S, Bonas U. 2009. How *Xanthomonas* type III effectors manipulate host plant. *Curr Opin in Microbiology* **12**:37-43.

Kay S, Hahn S, Marois E, Hause G, Bonas U. 2007. A bacterial effector acts as a plant transcription factor and induces a cell size regulator. *Science* **318**:648-651.

Kearney B, Staskawicz BJ. 1990. Widespread distribution and fitness contribution of *Xanthomonas campestris* avirulence gene avrBs2. *Nature* **346:**385-386.

Kaewnum S, Prathuangwong S, Burr TJ. 2006. A pectate lyase homolog, *xagP*, in *Xanthomonas axonopodis* pv. *glycines* is associated with hypersensitive response induction on tobacco. *Phytopathology* **96:**1230-1236.

Kim JF, Beer SV. 1998. HrpW of *Erwinia amylovora*, a new harpin that contains a domain homologous to pectate lyases of a distinct class. *J Bacteriol* **180:**5203-5210.

Kim JG, Li X, Roden JA, Taylor KW, Aakre CD, Su B, Lalonde S, Kirik A, Chen Y, Baranage G, McLane H, Martin GB, Mudgett MB. 2009a. *Xanthomonas* T3S effector XopN suppresses PAMP-triggered immunity and interacts with a tomato atypical receptor-like kinase and TFT1. *Plant Cell* **21:**1305-1323.

Kim SY, Kim JG, Lee BM, Cho JY. 2009b. Mutational analysis of the *gum* gene cluster required for xanthan biosynthesis in *Xanthomonas oryzae* pv *oryzae*. *Biotechnol*. *Lett*. **31**:265-270.

Kim JG, Taylor KW, Hotson A, Keegan M, Schmelz EA, Mudgett MB. 2008. XopD SUMO protease affects host transcription, promotes pathogen growth, and delays symptom development in *Xanthomonas* infected tomato leaves. *Plant Cell* **20:**1915-1929.

Kingsley MT, Gabriel DW*,* Marlow GC, Roberts P*.* 1993*.* The *opsX* Locus of *Xanthomonas campestris* affects host range and biosynthesis of lipopolysaccharide and extracellular polysaccharide*. J Bacteriol* **175***:*5839-5850*.*

Kiraly Z, El-Zahaby HM, Klement Z. 1997. Role of extracellular polysaccharide (eps) slime of plant pathogenic bacteria in protecting cells to reactive oxygen species. *Journal of Phytopathology* **145:**59-68.

Koebnik R, Kruger A, Thieme F, Urban A, Bonas U. 2006. Specific binding of the *Xanthomonas campestris* pv. *vesicatoria* AraC-type transcriptional activator HrpX to plant-inducible promoter boxes*. J Bacteriol* **188**:7652-7660.

Krylov VN. 2003. The role of horizontal gene transfer by bacteriophages in the origin of pathogenic bacteria. *Russian Journal of Genetics* **5**:483-504.

Laia ML, Moreira LM, Dezajacomo J, Brigati JB, Ferreira CB, Ferro MI, Silva AC, Ferro JA, Oliveira JC. 2009. New genes of *Xanthomonas citri* subsp. *citri* involved in pathogenesis and adaptation revealed by a transposon-based mutant library. *BMC Microbiol* **9:**12.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R et al. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* **23**(21):2947-2948.

Latreille P, Norton S, Goldman BS, Henkhaus J, Miller N, Barbazuk B, Bode HB, Darby C, Du Z, Forst S, Gaudriault S, Goodner B, Goodrich-Blair H, Slater S. 2007. Optical mapping as a routine tool for bacterial genome sequence finishing. *BMC Genomics* **8:**321.

Lee SW, Jeong KS, Han SW, Lee SE, Phee BK, Hahn TR, Ronald P. 2008. The *Xanthomonas oryzae* pv. *oryzae* PhoPQ two component system is required for AvrXA21 activity, *hrpG* expression and virulence. *J Bacteriol* **190**:2183-2197.

Li J, Wang N. 2011. The wxacO gene of *Xanthomonas citri* ssp. *citri* encodes a protein with a role in lipopolysaccharide biosynthesis, biofilm formation, stress tolerance and virulence. *Mol Plant Pathol* **12:**381-396.

Lin RH, Peng CW, Lin YC, Peng HL, Huang HC. 2011. The *xopE2* effector protein of *Xanthomonas campestris* pv. *vesicatoria* is involved in virulence and in the suppression of the hypersensitive response. *Botanical studies* **52:**55-72.

Lindeberg M. 2012. Genome-enabled prespectives on the composition, evolution and expression of virulence determinants in bacterial plant pathogens. *Phytopathology* **50**:111-132.

Lindgren PB, Peet RC, Panopoulos NJ. 1986. Gene cluster of *Pseudomonas syringae* pv. "*phaseolicola*" controls pathogenicity of bean plants and hypersensitivity of nonhost plants. *J Bacteriol* **168**:512-522.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}{}_{T}$ method. *Methods* **25**:402-408.

Lorenz C, Kirchner O, Egler M, Stuttmann J, Bonas U, Buttner D. 2008. HpaA from *Xanthomonas* is a regulator of type III secretion. *Mol Microbiol* **69**:344-360.

Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res* **25:**955-964.

Lu H, Patil P, Van Sluys MA, White FF, Ryan RP, Dow JM, Rabinowicz P, Salzberg SL, Leach JE, Sonti R, Brendel V, Bogdanove AJ. 2008. Acquisition and evolution of

plant pathogenesis-associated gene clusters and candidate determinants of tissue-specificity in *Xanthomonas*. *PLoS One* **3:**e3828.

Malamud F, Torres PS, Roeschlin R, Rigano LA, Enrique R, Bonomi HR, Castagnaro AP, Marano MR and Vojnov AA. 2011. The *Xanthomonas axonopodis* pv. *citri* flagellum is required for mature biofilm and canker development. *Microbiology* **157**:819-829.

Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen YJ, Chen Z, et al. 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437:**376-380.

McMullen M, Jones R, Gallenberg D. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. *Plant Disease* **81**:1340-1348.

Meyer A, Puhler A, Niehaus K. 2001. The lipopolysaccharides of the phytopathogen *Xanthomonas campestris* pv. *campestris* induce an oxidative burst reaction in cell cultures of Nicotiana tabacum. *Planta* **213**:214-222.

Metz M, Dahlbeck D, Morales CQ, Al Sady B, Clark ET, Staskawicz BJ. 2005. The conserved *Xanthomonas campestris* pv. *vesicatoria* effector protein XopX is a virulence factor and suppresses host defense in *Nicotiana benthamiana*. *Plant J* **41:**801-814.

Minsavage GV, Dahlbeck D, Whalen MC, Kearney B, Bonas U, Staskawicz BJ, Stall RE. 1990. Gene-for-gene relationships specifying disease resistance in *Xanthomonas campestris* pv. *vesicatoria*- pepper interactions. *Mol Plant Microbe Interact* **3**(1):41-47.

Mira A, Klasson L, Andersson SGE. 2002. Microbial genome evolution: sources of availability. *Curr Opin in Microbiology* **5**:506-512.

Moreira LM, Almeida NF, Potnis N, Digiampietri LA, Adi SS, Bortolossi JC, da Silva AC, da Silva AM, de Moraes FE, de Oliveira JC, et al. 2010. Novel insights into the genomic basis of citrus canker based on the genome sequences of two strains of *Xanthomonas fuscans* subsp. *aurantifolii*. *BMC Genomics* **11:**238.

Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nature Methods* **5**:621-628.

Mougous JD, Cuff ME, Raunser S, Shen A, Zhou M, Gifford CA, Goodman AL, Joachimiak G, Ordoñez CL, Lory S, Walz T, Joachimiak A, Mekalanos JJ. 2006. A virulence locus of *Pseudomonas aeruginos*a encodes a protein secretion apparatus. *Science* **312**:1526-1530.

Mukaihara T, Tamura N, Iwabuchi M. 2010. Genome-wide identification of large repertoire of *Ralstonia solanacearum* type III effector proteins by a new functional screen. *Mol Plant Microbe Interact* **23**:251-262.

Napoli C, Staskawicz B. 1987. Molecular characterization and nucleic acid sequence of an avirulence gene from race 6 of *Pseudomonas syringae* pv. *glycinea*. *J Bacteriol* **169:**572-578.

Newman MA, Dow JM, Molinaro A, Parrilli M. 2007. Priming, induction and modulation of plant defence responses by bacterial lipopolysaccharides. *J Endotoxin Res* **13**:69-84.

Newman MA, Von Roepenack E, Daniels M, Dow M. 2000. Lipopolysaccharides and plant responses to phytopathogenic bacteria. *Mol Plant Pathol* **1:**25-31.

Nimchuk ZL, Fisher EJ, Desveaux D, Chang JH, Dangl JL. 2007. The HopX (AvrPphE) family of *Pseudomonas syringae* type III effectors require a catalytic triad and a novel N-terminal domain for function. *Mol Plant Microbe Interact* **20:**346-357.

Noël L, Thieme F, Gäbler J, Büttner D, Bonas U. 2003. XopC and XopJ, two novel type III effector proteins from *Xanthomonas campestris* pv. *vesicatoria*. *J Bacteriol* **185**:7092-7102.

Noël L, Thieme F, Nennstiel D, Bonas U. 2001. cDNA-AFLP analysis unravels a genome-wide hrpG-regulon in the plant pathogen *Xanthomonas campestris* pv. *vesicatoria*. *Mol Microbiol* **41:**1271-1281.

Noël L, Thieme F, Nennstiel D, Bonas U. 2002. Two novel type III-secreted proteins of *Xanthomonas campestris* pv. *vesicatoria* are encoded within the hrp pathogenicity island. *J Bacteriol* **184:**1340-1348.

Nomura K, Melotto M, He S. 2005. Suppression of host defense in compatible plant-*Pseudomonas syringae* interactions. *Curr Opin Plant Biol* **8**:361-368.

Ochman H, Lawrence JG, Groisman EA. 2000. Lateral gene transfer and the nature of bacterial innovation. *Nature* **405:**299-304.

Palmer LE, McCombie WR. 2002. On the importance of being finished. *Genome Biol* **3**(10):comment2010.1-comment2010.4.

Park DS, Hyun JW, Park YJ, Kim JS, Kang HW, Hahn JH, Go SJ. 2006. Sensitive and specific detection of *Xanthomonas axonopodis* pv. *citri* by PCR using pathovar specific primers based on *hrpW* gene sequences. *Microbiol Res* **161**:145-149.

Pati A, Ivanova NN, Mikhailova N, Ovchinnikova G, Hooper SD, Lykidis A, Kyrpides NC. 2010. GenePRIMP: a gene prediction improvement pipeline for prokaryotic genomes. *Nat Methods* **7**(6):455-457.

Patil P, Bogdanove A, Sonti R. 2007. The role of horizontal transfer in the evolution of a highly variable lipopolysaccharide biosynthesis locus in xanthomonads that infect rice, citrus and crucifers. *BMC Evol Biol* **7:**243.

Petnicki-Ocwieja T, Schneider DJ, Tam VC, Chancey ST, Shan L, Jamir Y, Schechter LM, Janes MD, Buell CR, Tang X, Collmer A, Alfano JR. 2002. Genomewide identification of proteins secreted by the Hrp type III protein secretion system of *Pseudomonas syringae* pv. *tomato* DC3000. *Proc Natl Acad Sci USA* **99:**7652-7657.

Poplawsky AR, Chun W. 1997. *pigB* determines a diffusible factor needed for extracellular polysaccharide slime and xanthomonadin production in *Xanthomonas campestris* pv. *campestris*. *J Bacteriol* **179**:439-444.

Potnis N, Krasileva K, Chow V, Almeida NF, Patil PB, Ryan RP, Sharlach M, Behlau F, Dow JM, Momol M, et al. 2011. Comparative genomics reveals diversity among xanthomonads infecting tomato and pepper. *BMC Genomics* **12:**146.

Pukatzki S, McAuley SB, Miyata ST. 2009. The type VI secretion system: translocation of effectors and effector-domains. *Curr Opin Microbiol* **12**:11-17.

Pukatzki S, Ma AT, Sturtevant D, Krastins B, Sarracino D, Nelson WC, Heidelberg JF, Mekalanos JJ. 2006. Identification of a conserved bacterial protein secretion system in *Vibrio cholerae* using the *Dictyostelium* host model system. *Proc Natl Acad Sci* USA **103**:1528-1533.

Qian W, Jia Y, Ren SX, He YQ, Feng JX, Lu LF, Sun Q, Ying G, Tang DJ, Tang H, et al. 2005. Comparative and functional genomic analyses of the pathogenicity of phytopathogen *Xanthomonas campestris* pv. *campestris*. *Genome Research* **15**:757-767.

Rademaker JLW, Louws FJ, Schultz MH, Rossbach U, Vauterin L, Swings J, de Brujin FJ. 2005. A comprehensive species to strain taxonomic framework for *Xanthomonas*. *Phytopathology* **95**:1098-1111.

Rajeshwari R, Jha G, Sonti RV. 2005. Role of an in planta-expressed xylanase of *Xanthomonas oryzae* pv. *oryzae* in promoting virulence on rice. *Mol Plant Microbe Interact* **18:**830-837.

Ravin NV, Kuprianov VV, Gilcrease EB, Casjens SR. 2003. Bidirectional replication from an internal ori site of the linear N15 plasmid prophage. *Nucleic Acids Res* **31:**6552-6560.

Ray SK, Rajeshwari R, Sonti RV. 2000. Mutants of *Xanthomonas oryzae* pv. *oryzae* deficient in general secretory pathway are virulence deficient and unable to secrete xylanase. *Mol Plant Microbe Interact* **13:**394-401.

Records AR. 2011. The Type VI Secretion System: A Multipurpose Delivery System with a Phage-Like Machinery. *Mol Plant Microbe Interact* **24:**751-757.

Rigano L, Siciliano F, Enrique R, Sendín L, Filippone P, Torres P, Qüesta J, Dow J, Castagnaro A, Vojnov A, Marano M. 2007. Biofilm formation, epiphytic fitness,

and canker development in *Xanthomonas axonopodis* pv. *citri*. *Mol Plant Microbe Interact* **20:**1222-1230.

Roberts I. 1996. The biochemistry and genetics of capsular polysaccharide production in bacteria. *Annu Rev Microbiol* **50**:285-315.

Roden JA, Belt B, Ross JB, Tachibana T, Vargas J, Mudgett MB. 2004. A genetic screen to isolate type III effectors translocated into pepper cells during *Xanthomonas* infection. *Proc Natl Acad Sci USA* **101**:16624-16629.

Roine E, Wei W, Yuan J, Nurmiaho-Lassila EL, Kalkkinen N, Romantschuk M, He SY. 1997. Hrp pilus: an *hrp*-dependent bacterial surface appendage produced by *Pseudomonas syringae* pv. *tomato* DC3000. *Proc Natl Acad Sci* USA **94**:3459-3464.

Römling U, Gomelsky M, Galperin MY. 2005. C-di-GMP: the dawning of a novel bacterial signalling system. *Mol Microbiol* **57**:629-639.

Ronald P, Staskawicz B. 1988. The avirulence gene *avrBs1* from *Xanthomonas campestris* pv. *vesicatoria* encodes a 50-kD protein. *Mol Plant Microbe Interact* **1**:191-198.

Ryan R, Fouhy Y, Lucey J, Crossman L, Spiro S, He Y, Zhang L, Heeb S, Cámara M, Williams P, Dow J. 2006. Cell-cell signaling in *Xanthomonas campestris* involves an HD-GYP domain protein that functions in cyclic di-GMP turnover. *Proc Natl Acad Sci* USA **103**:6712-6717.

Ryan RP, Vorhölter FJ, Potnis N, Jones JB, Van Sluys MA, Bogdanove AJ, Dow JM. 2011. Pathogenomics of *Xanthomonas*: understanding bacterium-plant interactions. *Nat Rev Microbiol* **9**(5):344-355.

Rybak MA, Minsavage GV, Stall RE, and Jones JB. 2009. Identification of *Xanthomonas citri* ssp. *citri* host specificity genes in a heterologous expression host. *Molecular Plant Pathology* **10**(2):249-262.

Saddler GS, Bradbury JF. 2005. Family I: Xanthomonadaceae fam. nov. In *Bergey's Manual of Systematic Bacteriology*, *vol. 2 (The Proteobacteria), part B (The Gammaproteobacteria),* 2nd ed. (ed. Brenner DJ, Krieg NR, Staley, JT, Garrity GM), pp. 63-90. Springer, New York, New York.

Salomon D, Dar D, Sreeramulu S, Sessa G. 2011. Expression of *Xanthomonas campestris* pv. *vesicatoria* type III effectors in yeast affects cell growth and viability. *Mol Plant Microbe Interact* **24:**305-314.

Sandkvist M. 2001. Biology of type II secretion. *Mol Microbiol* **40**:271-283.

Schaad NW, Postnikova E, Lacy G, Sechler A, Agarkova I, Stromberg PE, Stromberg VK, Vidaver AK. 2006. Emended classification of xanthomonad pathogens on citrus. *Syst Appl Microbiol* **29:**690-695.

Schneider DJ, Collmer A. 2010. Studying plant-pathogen interactions in the genomics era: beyond molecular Koch's postulates to systems biology. *Annu Rev Phytopathol* **48**:457-479.

Schoulties CL, Civerolo EL, Miller JW, Stall RE. 1987. Citrus canker in Florida. *Plant Disease* **71**:388-395.

Schubert TS. 1991. Recent history of citrus canker eradication programs in Florida. *Newsletter of the Florida Phytopathological Society* **2**:1-6.

Schubert TS, Rizvi SA, Sun X, Gottwald TR, Graham JH, Dixon WN. 2001. Meeting the challenge of eradicating citrus canker in Florida - Again. *Plant Disease* **85**:340-356.

Schulte R, Bonas U. 1992. A *Xanthomonas* pathogenicity locus is induced by sucrose and sulfur-containing amino acids. *The Plant Cell* **4**:79-86.

Shiotani H, Fujikawa T, Ishihara H, Tsuyumu S, Ozaki K. 2007. A *pthA* homolog from *Xanthomonas axonopodis* pv. *citri* responsible for host-specific suppression of virulence. *J Bacteriol* **189:**3271-3279.

Shrivastava S, Mande SS. 2008. Identification and functional characterization of gene components of type VI secretion system in bacterial genomes. *PLoS One* **3**:e2955.

Siciliano F, Torres P, Sendín L, Bermejo C, Filippone P, Vellice G, Ramallo J, Castagnaro A, Vojnov A, Marano MR. 2006. Analysis of the molecular basis of *Xanthomonas axonopodis* pv. *citri* pathogenesis in *Citrus limon*. *Electronic Journal of Biotechnology* **9:**199-204.

Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M. 2006. ISfinder: the reference centre for bacterial insertion sequences. *Nucleic Acids Res* **34:**D32-D36.

Simm R, Morr M, Kader A, Nimtz M, Römling U. 2004. GGDEF and EAL domains inversely regulate cyclic di-GMP levels and transition from sessility to motility. *Mol Microbiol* **53**:1123-1134.

Simpson AJ, Reinach FC, Arruda P, Abreu FA, Acencio M, Alvarenga R, Alves LM, Araya JE, Baia GS, Baptista CS, et al. 2000. The genome sequence of the plant pathogen *Xylella fastidiosa*. The *Xylella fastidiosa* Consortium of the Organization for Nucleotide Sequencing and Analysis. *Nature* **406:**151-159.

Slater H, Alvarez-Morales A, Barber CE, Daniels MJ, Dow JM. 2000. A two-component system involving an HD-GYP domain protein links cell-cell signalling to

pathogenicity gene expression in *Xanthomonas campestris*. *Mol Microbiol* **38:**986-1003.

Stall RE, Civerolo EL. 1991. Research relating to the recent outbreak of citrus canker in Florida. *Annu Rev Phytopathol* **29**:399-420.

Sun, M. 1984. The Mystery of Florida's Citrus Canker: Scientists haven't seen this bacterial strain before, but it's already led to the destruction of millions of seedlings. *Science* **226:**322-323.

Sun X, Stall RE, Jones JB, Cubero J, Gottwald TR, Graham JH, Dixon WN, Schubert TS, Chaloux PH, Stromberg VK et al. 2004. Detection and characterization of a new strain of citrus canker bacteria from Key/Mexican Lime and Alemow in South Florida. *Plant Disease* **88**(11):1179-1188.

Swafford DL. 2003. PAUP*. Phylogenetic analysis using parsimony (*and other methods). 4.0 ed. Sinauer associates, Sunderland, Massachusetts.

Swarup S, Feyter DR, Brlansky RH, Gabriel DW. 1991. A pathogenicity locus from *Xanthomonas citri* enables strains from several pathovars of *Xanthomonas campestris* to elicit canker-like lesions on citrus. *Phytopathology* **81**:802-809.

Swarup S, Yang Y, Kingsley MT, Gabriel DW. 1992. An *Xanthomonas citri* pathogenicity gene, *pthA*, pleiotropically encodes gratuitous avirulence on nonhosts. *Mol Plant Microbe Interact* **5:**204-213.

Swords KM, Dahlbeck D, Kearney B, Roy M, Staskawicz BJ. 1996. Spontaneous and induced mutations in a single open reading frame alter both virulence and avirulence in *Xanthomonas campestris* pv. *vesicatoria avrBs2*. *J Bacteriol* **178:**4661-4669.

Szczesny R, Jordan M, Schramm C, Schulz S, Cogez V, Bonas U, Büttner D. 2010. Functional characterization of the Xcs and Xps type II secretion systems from the plant pathogenic bacterium *Xanthomonas campestris* pv *vesicatoria*. *New Phytol* **187:**983-1002.

Szurek B, Rossier O, Hause G, Bonas U. 2002. Type III-dependent translocation of the *Xanthomonas* AvrBs3 protein into the plant cell. *Mol Microbiol* **46:**13-23.

Tang JL, Liu YN, Barber CE, Dow JM, Wootton JC, Daniels MJ. 1991. Genetic and molecular analysis of a cluster of *rpf* genes involved in positive regulation of synthesis of extracellular enzymes and polysaccharide in *Xanthomonas campestris* pathovar *campestris*. *Mol Gen Genet* **226**:409-417.

Thieme F. 2008. Genombasierte Identifizierung neuer potentieller Virulenzfaktoren von *Xanthomonas campestris* pv. *vesicatoria*. Mathematisch-Naturwissenschaftlich-Technische Fakultät der Martin-Luther Universität, Halle-Wittenberg.

Thieme F, Koebnik R, Bekel T, Berger C, Boch J, Büttner D, Caldana C, Gaigalat K, Goesmann A, Kay S, et al. 2005. Insights into genome plasticity and pathogenicity of the plant pathogenic bacterium *Xanthomonas campestris* pv. *vesicatoria* revealed by the complete genome sequence. *J Bacteriol* **187:**7254-7266.

Thieme F, Szczesny R, Urban A, Kirchner O, Hause G, Bonas U. 2007. New type III effectors from *Xanthomonas campestris* pv. *vesicatoria* trigger plant reactions dependent on a conserved N-myristoylation motif. *Mol Plant Microbe Interact* **20:**1250-1261.

Timmer LW, Gottwald TR, Zitko SE. 1991. Bacterial exudation from lesions of asiatic citrus canker and citrus bacterial spot. Plant Disease **75:**4.

Tischler AD, Camilli A. 2004. Cyclic diguanylate (c-di-GMP) regulates *Vibrio cholerae* biofilm formation. *Mol Microbiol* **53**:857-869.

Torres PS, Malamud F, Rigano LA, Russo DM, Marano MR, Castagnaro AP, Zorreguieta A, Bouarab K, Dow JM, Vojnov AA. 2007. Controlled synthesis of the DSF cell-cell signal is required for biofilm formation and virulence in *Xanthomonas campestris*. *Environ Microbiol* **9**:2101-2109.

Tseng TT, Tyler BM, Setubal JC. 2009. Protein secretion systems in bacterial-host associations, and their description in the Gene Ontology. *BMC Microbiol* **9**:Suppl 1:S2.

Tsuda K, Katagiri F. 2010. Comparing signal mechanisms engaded in pattern-triggered and effector-triggered immunity. *Curr Opinion Plant Biol* **13**:459-465.

Tyson GW, Chapman J, Hugenholtz P, Allen EE, Ram RJ, Richardson PM, Solovyev VV, Rubin EM, Rokhsar DS, Banfield DF. 2004. Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature* **428:**37-43.

Van der Plank JE. 1963. *Plant Diseases: Epidemics and control*. Academic Press, New York, USA.

Vauterin L, Hoste B, Kersters K, Swings J. 1995. Reclassification of *Xanthomonas*. *International Journal of Systematic Bacteriology* **45**(3):472-489.

Vauterin L, Rademaker J, Swings J. 2000. Synopsis on the taxonomy of the genus *Xanthomonas*. *Phytopathology* **90:**677-682.

Verniere CJ, Gottwald TR, and Pruvost O. 2002. Disease development and symptom expression of *Xanthomonas campestris pv. citri* in various citrus plant tissues. *Phytopathology* **93**:832-843.

Verniere C, Hartung JS, Pruvost OP, Civerolo EL, Alvarez AM, Maestri P, Luisetti J. 1998. Characterization of phenotypically distinct strains of *Xanthomonas axonopodis* pv. *citri* from Southwest Asia. *European Journal of Plant Pathology* **104**:477-487.

Wang LH, He Y, Gao Y, Wu JE, Dong YH, He C, Wang SX, Weng LX, Xu JL, Tay L, Fang RX, Zhang LH. 2004. A bacterial cell-cell communication signal with cross-kingdom structural analogues. *Mol Microbiol* **51**:903-912.

Wang L, Leach JE, Bogdanove AJ. 2006. Genetic background of *Xanthomonas oryzae* pv. *oryzae* strains influences the function of specific avirulence genes. *Phytopathology* **96**:S121.

Wei CF, Kvitko BH, Shimizu R, Crabill E, Alfano JR, Lin NC, Martin GB, Huang HC, Collmer A. 2007. A *Pseudomonas syringae* pv. *tomato* DC3000 mutant lacking the type III effector HopQ1-1 is able to cause disease in the model plant *Nicotiana benthamiana*. *Plant J* **51:**32-46.

Wengelnik K, Bonas U. 1996a. HrpXv, an AraC-type regulator, activates expression of five of the six loci in the *hrp* cluster of *Xanthomonas campestris* pv. *vesicatoria*. *Mol Plant Microbe Interact* **12**:3462-3489.

Wengelnik K, Van Den Ackerveken G, Bonas U. 1996b. HrpG a key *hrp* regulatory protein of *Xanthomonas campestris* pv. *vesicatoria* is homologous to two-component response regulators. *Mol Plant Microbe Interact* **9**:704-712.

Welch RA. 1991. Pore-forming cytolysins of gram-negative bacteria. *Mol Microbiol* **5**:521-528.

White FF, Potnis N, Jones JB, Koebnik R. 2009. The type III effectors of *Xanthomonas*. *Mol Plant Pathol* **10:**749-766.

Williams AW, Yamaguchi S, Togashi F, Aizawa SI, Kawagishi I, Macnab RM. 1996. Mutations in *fliK* and *flhB* affecting flagellar hook and filament assembly in *Salmonella typhimurium*. *J Bacteriol* **178:**2960-2970.

Yan Q, Wang W. 2012. High-throughput screening and analysis of genes of *Xanthomonas citri* subsp. *citri* involved in citrus canker symptom development. *Mol Plant Microbe Interact* **25**:69-84.

Yang B, White FF. 2004. Diverse members of the AvrBs3/PthA family of type III effectors are major virulence determinants in bacterial blight disease of rice. *Mol Plant Microbe Interact* **17:**1192-1200.

Yen MR, Peabody CR, Partovi SM, Zhai Y, Tseng YH, Saier MH. 2002. Protein-translocating outer membrane porins of Gram-negative bacteria. *Biochim Biophys Acta* **1562**:6-31.

Yeo HJ, Waksman G. 2004. Unveiling molecular scaffolds of the type IV secretion system. *J Bacteriol* **186**:1919-1926.

Yun MH, Torres PS, El Oirdi M, Rigano LA, Gonzalez-Lamothe R, Marano MR, Castagnaro AP, Dankert MA, Bouarab K, Vojnov AA. 2006. Xanthan induces plant susceptibility by suppressing callose deposition. *Plant Physiol* **141**:178-187.

Zhao B, Ardales EY, Raymundo A, Bai J, Trick HN, Leach JE, Hulbert SH. 2004. The *avrRxo1* gene from the rice pathogen *Xanthomonas oryzae* pv. *oryzicola* confers a nonhost defense reaction on maize with resistance gene *Rxo1*. *Mol Plant Microbe Interact* **17:**771-779.

BIOGRAPHICAL SKETCH

Neha Jalan was born in Mumbai, Maharashtra, India in 1983. She obtained her bachelor's degree in microbiology in 2003 from Mithibai College, University of Mumbai, India. She continued to pursue her master's degree at University of Mumbai. During this time she worked on treatment of starchy wastewater by amylase producing soil isolates. She completed her master's degree in 2005 and joined Sophia College, University of Mumbai as a lecturer in industrial microbiology. During this time she co-wrote a grant and was awarded to do research on microbial degradation of methyl parathion: characterization of its metabolites and their effects on biological system. In November 2006 she started working as Junior research fellow jointly at Bhavan's College, University of Mumbai and ISOMED, Bhabha Atomic Research Center. The research focused on production of fructooligosaccharide by gamma radiation processing of microbial levan and radiation effect studies on its use as prebiotic. In August 2008, she joined the Ph.D. program in the Department of Microbiology and Cell Science at the University of Florida. She worked under the guidance of Dr. Nian Wang focusing her research on comparative genomics and transcriptomics of host-specific xanthomonads causing citrus canker and citrus bacterial spot. As a graduate student she presented her work at departmental symposiums and 2010-2012 American Phytopathological Society annual meetings. She was awarded the 2nd best oral and poster presentation award at the department of MCS graduate student symposium in 2010 and 2012, respectively. Neha is a member of American Phytopathological Society (APS) and American Society of Microbiology (ASM).