Molecular Plant Pathology

MOLECULAR PLANT PATHOLOGY (2018) **19**(8), 2036–2050 DOI: 10.1111/mpp.12678

Review

Clavibacter michiganensis ssp. *michiganensis*: bacterial canker of tomato, molecular interactions and disease management

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SUMMARY

Bacterial canker disease is considered to be one of the most destructive diseases of tomato (*Solanum lycopersicum*), and is caused by the seed-borne Gram-positive bacterium *Clavibacter michiganensis* ssp. *michiganensis* (*Cmm*). This vascular pathogen generally invades and proliferates in the xylem through natural openings or wounds, causing wilt and canker symptoms. The incidence of symptomless latent infections and the invasion of tomato seeds by *Cmm* are widespread. Pathogenicity is mediated by virulence factors and transcriptional regulators encoded by the chromosome and two natural plasmids. The virulence factors include serine proteases, cell wall-degrading enzymes (cellulases, xylanases, pectinases) and others. Mutational analyses of these genes and gene expression profiling (via quantitative reverse transcription-polymerase chain reaction, transcriptomics and proteomics) have begun to shed light on their roles in colonization and virulence, whereas the expression of tomato genes in response to *Cmm* infection suggests plant factors involved in the defence response. These findings may aid in the generation of target-specific bactericides or new resistant varieties of tomato. Meanwhile, various chemical and biological controls have been researched to control *Cmm*. This review presents a detailed investigation regarding the pathogen *Cmm*, bacterial canker infection, molecular interactions between *Cmm* and tomato, and current perspectives on improved disease management.

Keywords: crop disease, foliar infection, genome, *Microbacteriaceae*, molecular biology, plant–microbe interactions, systemic infection.

INTRODUCTION

Tomato is the world's largest vegetable crop with an annual production at 169 million tons, representing 16% of total vegetable production and 58 billion US dollars [Food and Agricultural Organization (FAO), 2016]. Bacterial canker of tomato, caused by *Clavibacter michiganensis* ssp. *michiganensis* (*Cmm*), is considered to be a serious threat to the processing and fresh market tomato industries, having caused catastrophic epidemics in most tomato-growing areas of the world (Blank *et al.*, 2016; Kleitman *et al.*, 2008; Smith, 1910; Volcani, 1985).

Cmm is a non-motile, Gram-positive actinomycete, and the only recognized species of the genus *Clavibacter*. It has been grouped into several subspecies which cause devastating diseases in agricultural crops (Table 1; Davis *et al.*, 1984), although digital DNA–DNA hybridization (dDDH) and average nucleotide identity (ANI) suggest that the subspecies fit the criteria for separate species (Tambong, 2017).

Plants infected with *Cmm* show various symptoms depending on the age of the host plant, cultivar susceptibility and virulence of *Cmm* (Gleason *et al.*, 1993), together with certain environmental conditions, including temperature and humidity (de León *et al.*, 2011). When plants are infected at early stages of their life (as seeds or young seedlings), they develop systemic infections (also called primary infections) that affect fruit quality and yield, and typically lead to plant death. In contrast, older plants usually develop foliar infections (also called secondary infections), which cause chlorosis of leaves, but may or may not affect the quality and yield of the current crop.

During systemic infection, plants can at first appear asymptomatic, with symptoms showing after 6–8 weeks as the bacterium multiplies to a high titre (Gitaitis *et al.*, 1991), reaching 10^9–10^{10} per gram of plant tissue homogenate (Meletzus *et al.*, 1993). *Cmm* invades and proliferates in xylem vessels, causing browning of the internal vasculature, with the gradual degradation of vascular tissues (Fig. 1). This interferes with water transport and leads to wilting during early stages of infection (Eichenlaub and Gartemann, 2011). Tomato plants that are infected during late developmental stages may be asymptomatic or have a slow wilting process (Gitaitis *et al.*, 1991; Sharabani *et al.*, 2013). They can survive infection and may produce marketable fruit, but can become an efficient source of infection for the successive growing season (Sharabani *et al.*, 2013).

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Table 1 *Clavibacter michiganensis* subspecies and hosts

Subspecies	Common name or symptoms	Major hosts	Reference
californiensis	Asymptomatic	Tomato	Yasuhara-Bell and Alvarez (2015)
capsici	Bacterial canker disease	Bell pepper; sweet pepper	Oh et al. (2016)
chilensis	Asymptomatic	Tomato	Yasuhara-Bell and Alvarez (2015)
insidiosus	Wilting and stunting	Alfalfa	McCulloch (1925)
michiganensis	Bacterial wilt and canker	Tomato	Davis et al. (1984); Strider (1969)
nebraskensis	Wilt and blight disease	Maize	Vidaver and Mandel (1974)
phaseoli	Leaf yellowing	Common bean	Gonzalez and Trapiello (2014)
sepedonicus	Ring rot disease	Potato	Manzer and Genereux (1981)
tessellarius	Leaf freckles and leaf spot	Wheat	Carlson and Vidaver (1982)

The effects of *Cmm*, including failure of tomato production and premature death of the entire plant, can result in substantial economic losses (Chang *et al.*, 1992). Field experiments have recorded yield losses of up to 84% in commercial fields of Ontario, Canada (Poysa, 1993), 20%–30% in France (Rat *et al.*, 1991) and 46% in Illinois, USA (Chang *et al.*, 1992), although the occurrence of this disease is sporadic and losses caused by outbreaks can also be minor.

Few Gram-positive phytopathogens have been studied in detail to explore the mechanisms utilized by bacteria to sense the host plant, colonize and counteract plant defence responses. However, the publication of *Cmm* genome sequences has provided an excellent platform for molecular investigations into disease induction and host–pathogen interactions (Gartemann *et al.*, 2008), providing insights into the mechanisms of *Cmm* virulence and the defence responses of tomato.

This review illustrates the current knowledge of bacterial canker disease, the global analysis approaches used to dissect *Cmm*–tomato molecular interactions, together with disease management.

CMM TRANSMISSION

Infected seeds are responsible for the long-distance transmission and dissemination of *Cmm*, enabling its introduction to previously disease-free regions (Fatmi *et al.*, 1991). Genetic diversity amongst *Cmm* isolates from narrow geographical ranges (Kleitman *et al.*, 2008; Milijašević-Marčić *et al.*, 2012; Tancos *et al.*, 2015; Thapa *et al.*, 2017; Wassermann *et al.*, 2017) suggests that the introduction of foreign strains is common, as is their ability to adapt to the new habitat (Jacques *et al.*, 2012).

Seed infection occurs when *Cmm* enters the seed coat and endosperm through the vascular system of an infected female

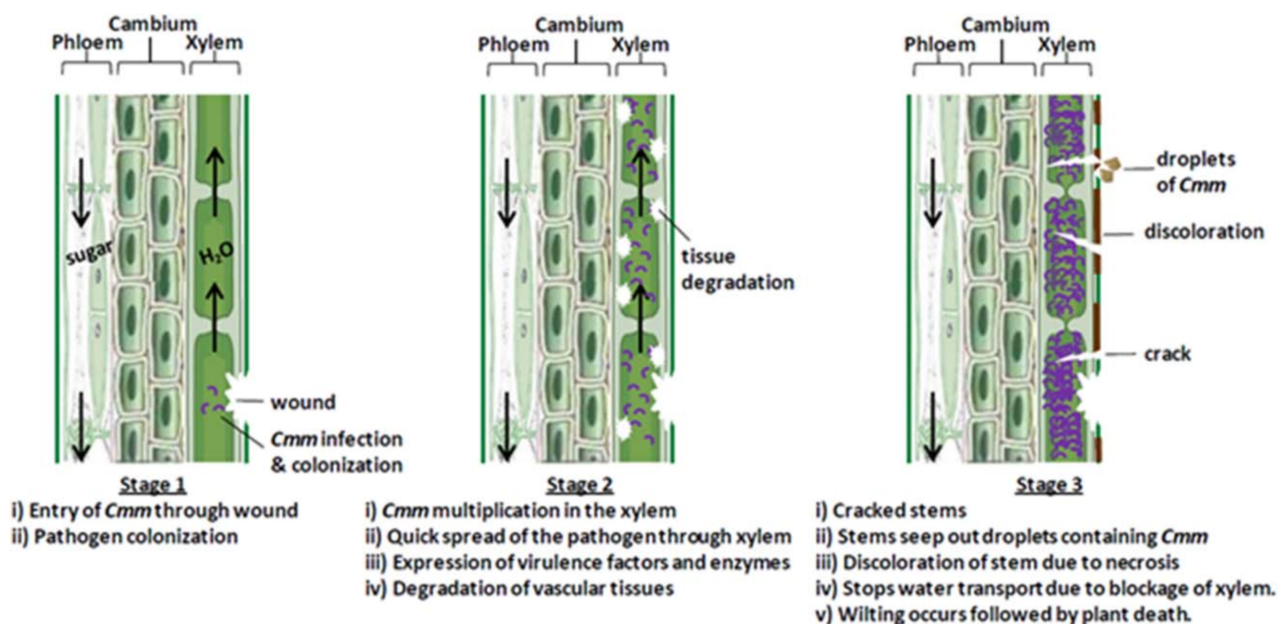

Fig. 1 Schematic diagram showing the stages of bacterial canker disease progression in tomato stem following *Clavibacter michiganensis* ssp. *michiganensis* (*Cmm*) infection.

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parent (Fatmi and Schaad, 1988). Seeds that are contaminated externally with *Cmm*, through contact with other sources of the bacterium, can also serve as the initial source of inoculum for systemic infections (Quesada-Ocampo *et al.*, 2012). Contaminated soil and plant debris are also important sources of systemic infections (Hadas *et al.*, 2005). *Cmm* can survive in plant debris under natural field conditions in the USA for more than 10 months (Fatmi and Schaad, 2002), and may contribute to soil-borne infections for up to 4 years (Ciccarone and Carilli, 1948, as cited in Fatmi and Schaad, 2002).

Cmm enters leaves, stems and roots through wounds or natural openings, including stomata and hydathodes (Carlton *et al.*, 1998; Tancos *et al.*, 2013), allowing the disease to spread laterally via splashing or dripping water or from contaminated tools (Carlton *et al.*, 1998; Chang *et al.*, 1991; Gitaitis *et al.*, 1991; Ricker and Riedel, 1993; Xu *et al.*, 2010).

As *C. michiganensis* subspecies that are pathogenic in a particular plant host can endophytically colonize non-host plants, the disease may also be disseminated as endophytes associated with non-host plants (Thapa *et al.*, 2017). Previous reports of bacterial and fungal endophytes have demonstrated similar patterns (Darrasse *et al.*, 2010; Ploch and Thines, 2011; Sowley *et al.*, 2010).

MOLECULAR BIOLOGY OF *CMM* PATHOGENESIS

Genomic features of *Cmm*

Sequenced genomes of *Cmm* tomato pathogen strains include a chromosome of 3.3–3.6 Mbp and a plasmid, pCM1, of 31–59 kb. Most of these strains have a second plasmid, pCM2, of 64–109 kb, which, compared with pCM1, is less conserved amongst tomato pathogenic strains in both size and gene content. By contrast, both plasmids are often absent in tomato endophytic strains of *C. michiganensis*, some of which cause disease in other plant species (Thapa *et al.*, 2017). Although much genetic research has been conducted with the wild-type *Cmm* strain NCPPB382 (*Cmm382*), other strains have been sequenced more recently (Gartemann *et al.*, 2008; Thapa *et al.*, 2017).

Cmm belongs to the phylum *Actinobacteria* which is characterized as having genomes with high GC content. However, there are about 20 regions identified in the *Cmm382* genome with low GC content (Gartemann *et al.*, 2008), many of which are involved in pathogenesis. In particular, a 129-kb low GC region near the origin of replication is recognized as a part of the chromosomal *chp*/*tomA* pathogenicity island (PAI) (Gartemann *et al.*, 2008). The *chp*/*tomA* PAI is the only genomic island present in all 12 sequenced *Cmm* strains that are pathogenic in tomato, whereas it is absent in the five sequenced tomato endophyte strains of *C. michiganensis* (Thapa *et al.*, 2017). Within this PAI, the *chp* subregion contains putative protease-encoding genes, and other genes known to be involved in virulence, whereas the *tomA* subregion contains *tomA*, encoding tomatinase, which is involved in the degradation of tomatin and provides basal defence to tomato (Kaup *et al.*, 2005; Stork *et al.*, 2008).

Virulence genes

Although the precise functions and interactions of *Cmm* virulence genes remain unknown, expression studies and the effects of mutations on disease progression have begun to reveal the general roles of some genes. As a number of putative virulence factors are present on the two plasmids, pCM1 and pCM2, and on the *chp*/*tomA* PAI, several studies have utilized natural variants or derivative strains that lack these specific loci. Strain *Cmm*27, a deletion mutant of *Cmm*382 that lacks the *chp*/*tomA* PAI, exhibits reduced colonization of tomato compared with *Cmm*382, and does not produce disease symptoms (Chalupowicz *et al.*, 2010). Other derivatives of *Cmm*382 include *Cmm*101, *Cmm*102 and *Cmm*100, which lack the plasmids pCM2, pCM1 or both, respectively. *Cmm*100 will colonize plants without producing a wilting phenotype (although it does induce localized leaf blisters after foliar infection), and is therefore considered to be non-virulent. *Cmm*101 and *Cmm*102 both exhibit reduced virulence compared with *Cmm*382, but remain pathogenic (Chalupowicz *et al.*, 2017).

Similar to *Cmm*100 (which lacks pCM1 and pCM2), a plasmid-free derivative of strain *Cmm*CASJ002 colonizes plants with titres similar to its parent strain, but does not elicit tomato canker symptoms. Similar to *Cmm*101 (which lacks pCM2), a derivative of *Cmm*CASJ002 that lacks pCM2, but retains pCM1, exhibits reduced wilting and weak pathogenicity, but colonizes plants with titres similar to the parent strain. However, *Cmm*CASJ001 and *Cmm*CASJ007 are both wild-type isolates that naturally lack pCM2 and yet retain pathogenicity, with virulence no lower than that of *Cmm*382. Removing pCM1 from *Cmm*CASJ001 does, however, eliminate tomato canker symptoms and produce five-fold lower bacterial titres compared with the parent strain (Thapa *et al.*, 2017).

It seems therefore that pCM1 is a major contributing factor to virulence, whereas the role of pCM2 is strain dependent (Thapa *et al.*, 2017). Nevertheless, most tomato-pathogenic *Cmm* strains harbour both plasmids (e.g. nine of 11 Californian isolates; Thapa *et al.*, 2017) or genes normally associated with both plasmids, as determined by polymerase chain reaction (PCR) (e.g. 46 of 51 New York isolates, and 12 of 12 Argentinian isolates; Tancos *et al.*, 2015; Wassermann *et al.*, 2017). It is therefore possible that the requirement for pCM2 in the pathogenicity of some *Cmm* strains, but not others, stems from the integration of pCM2-associated genes into the chromosome. In support of this concept, *C. michiganensis* ssp. *nebraskensis*, which does not harbour plasmids, encodes on its chromosome homologues of 28 genes which are present on plasmids in other *C. michiganensis* subspecies (Tambong, 2017). Although PCR-based detection failed to find

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plasmid-associated genes in some isolates of *Cmm*, this method may be limited by overly specific primers that are unable to hybridize with more divergent sequences.

Of the specific genes associated with virulence that are found on each plasmid, on the *chp*/*tomA* PAI and at other chromosomal locations (Table 2), the majority encode proteases and other plant cell wall-degrading enzymes (Burger *et al*., 2005; Savidor *et al*., 2012). Tomatinase, encoded on the *chp*/*tomA* PAI, is also an important contributor to virulence.

Tomatinase

Encoded on the *chp*/*tomA* PAI, tomatinase (Tom) is amongst 13 predicted secreted proteins that are common to all *Cmm* tomato pathogens (Tancos *et al*., 2015; Thapa *et al*., 2017), with the possible exception of *Cmm*NCPPB3264 (by Southern blot; Kaup *et al*., 2005). TomA degrades the tomato alkaloid α-tomatine, which is implicated in plant host defence against microbial pathogens. However, a *tomA* insertional mutant of *Cmm*101 retained similar virulence and bacterial titres to *Cmm*101, suggesting that *tomA* is not a virulence factor (Kaup *et al*., 2005). Nevertheless, as the comparison was made using *Cmm*101, a pCM2-free derivative which already shows reduced virulence compared with the parent strain *Cmm*382, it is possible that *tomA* may contribute to the virulence of *Cmm*. A study of *Fusarium oxysporum* f. sp. *lycopersici*, a fungal pathogen of tomato, found that tomatinase is required for full virulence, although it is not essential for pathogenicity (Pareja-Jaime *et al*., 2008).

Serine proteases

Putative virulence-associated *Cmm* proteases are encoded by the plasmids, the *chp*/*tomA* PAI and other chromosomal loci (Table 2; Fig. 2), and are members of three distinct families. They are secreted proteins whose substrates are not yet known. Nonetheless, these degradative enzymes might facilitate bacterial colonization via the degradation and alteration of host cell physiology to ultimately acquire nutrients and manipulate the host defence responses.

Proteases are implicated in various plant–microbe interactions. An extracellular protease (ecpAXoc) from *Xanthomonas oryzae* pv. *oryzicola*, a bacterial pathogen of rice, induces chlorosis- and necrosis-like symptoms when injected into rice leaves, indicating that it is a virulence factor (Zou *et al*., 2012). In *Fusarium oxysporum* f. sp. *lycopersici*, a causative agent of Fusarium wilt in tomato, the secreted serine protease FoSep1 cleaves extracellular tomato chitinases, which are host defensive enzymes that target fungal cell walls (Jashni *et al*., 2015). In contrast, the secreted serine protease PrtA of the Gram-negative bacterial pathogen of grapevines, *Xylella fastidiosa* strain Temecula1, reduces biofilm formation and is classified as an antivirulence factor, which, when disrupted, results in a hypervirulent phenotype (Gouran *et al*., 2016). Antivirulent factors may include proteins whose expression is advantageous outside of the host, but detrimental within the host, and therefore its expression is eventually lost or differentially regulated via evolution (Bliven and Maurelli, 2012). Thus, although not all proteases involved in plant–microbe interactions are necessarily virulence factors, those which are antivirulence factors are expected to be less common or expressed at lower levels in pathogenic *Cmm*.

Pat-1 family. The Chp or Pat-1 family of serine proteases is encoded by three genes on plasmid pCM2 (*pat-1*, *phpA* and *phpB*) and seven genes on the *chp*/*tomA* PAI (*chpA*–*chpG*; Burger *et al*., 2005). Most of the tested Chp family genes (*chpA*, *chpC*, *chpF*, *chpG* and *phpA*, but not *phpB*) produce up-regulated transcripts in minimal medium compared with medium supplemented with plant tissue homogenate (Flügel *et al*., 2012). Expression of *pat-1*, *chpC*, *chpE*, *chpF* and *chpG* is detected at the transcript and/or protein level in systemically infected plants, and the expression of *chpC* is detected during foliar infection (Chalupowicz *et al*., 2010, 2017; Savidor *et al*., 2012).

Introduction of plasmid-encoded *pat-1* into *Cmm*100, a derivative of strain *Cmm*382 that lacks both plasmids, restores some level of virulence and results in the wilting of infected plants (Meletzus *et al*., 1993), whereas simultaneous introduction of both *phpA* and *phpB* into *Cmm*100 does not produce wilting symptoms in tomato (Burger *et al*., 2005). Therefore, *pat-1* appears to be the most and possibly only impactful virulence gene on the pCM2 plasmid. Although some pathogenic *Cmm* naturally lack pCM2 (Thapa *et al*., 2017), it would be interesting to determine whether such strains possess a *pat-1* orthologue elsewhere in the genome.

The remaining Chp family proteases are clustered in a region of about 50 kb on the PAI of *Cmm382* (Stork *et al*., 2008). All of these tested chromosomal genes (*chpC*, *chpE*, *chpF* and *chpG*), when mutated, show a lower severity of leaf blisters during foliar infection (Chalupowicz *et al*., 2017). Mutation of *chpC* also leads to a dramatic reduction of *in planta* titre, followed by weak disease symptoms (Stork *et al*., 2008), including a significantly reduced incidence of leaf blisters during foliar infection and significantly reduced incidence of wilting during systemic infection (Chalupowicz *et al*., 2017). It is therefore implicated in both colonization and virulence.

Plant gene expression is also affected by *chpC*. Compared with plants infected with the *Cmm*382 wild-type strain, tomato plants infected with a Δ*chpC* mutant strain show increased levels of transcripts encoding 1-aminocyclopropane-1-carboxylic acid oxidase 1 (ACO1) and pathogenesis-related protein 4 (PR4) during blister formation (Chalupowicz *et al*., 2017). ACO1 is an enzyme involved in the biosynthesis of ethylene, a plant hormone with roles in development and defence, which is normally up-regulated in *Cmm*-infected plants (Savidor *et al*., 2012). Tomato plants that are insensitive to ethylene or deficient in its production experience

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Table 2 Locations and summary of mutant and expression studies for putative virulence genes of *Clavibacter michiganensis* ssp. *michiganensis*.

			Time of expression (hpi)			
Gene	Location	Expression in minimal medium†	Systemic infection	Foliar infection	Results from genetic studies	Suspected role
Chp family proteases						
chpA*	PAI	Yes				
chpB*	PAI					
chpC	PAI	Yes	72‡	8‡	Mutant has reduced incidence of plants with foliar blisters, reduced incidence of wilting (Chalupowicz et al., 2017), reduction of *in planta* titre and weak disease symptoms (Stork et al., 2008)	Colonization, virulence
chpD*	PAI					
chpE	PAI		192§			
chpF	PAI	Yes	192§			
chpG	PAI	Yes	192§		No effect on systemic colonization or virulence, abolished HR in non-host plants (Lu et al., 2015; Stork et al., 2008)	
pat-1	pCM2		24, 48‡, 12, 24, 72, 96, 168¶, 192§		Induces virulence when cloned into *Cmm*100 (Meletzus et al., 1993).	Virulence
phpA	pCM2	Yes			Does not induce virulence when cloned into *Cmm*100 (Burger et al., 2005)	
phpB	pCM2	No			Does not induce virulence when cloned into *Cmm*100 (Burger et al., 2005)	
Chymotrypsin-related serine proteases						
ppaA	PAI	Yes				
ppaB1	PAI	Yes	192§, **			
ppaB2	PAI	Yes	192§, **			
ppaC	PAI	Yes	192§			
ppaD	PAI	Yes	192§			
ppaE	PAI	No				
ppaF	Other	No				
ppaG	Other	No	192§			
ppaH	Other	Yes	192§			
ppaI	Other	No	192§			
ppaJ	pCM1	Yes				
Subtilase proteases						
sbtA	PAI	No		16‡	Mutant has reduced incidence of foliar blisters, less wilting (Chalupowicz et al., 2017)	Virulence
sbtB	Other	No	Early††			
sbtC	Other	No	192§			
Cellulases						
celA	pCM1	Yes	8, 16, 24, 28‡, 12, 24, 72, 96, 168¶, 192§	Not detected at ≥ four-fold	Mutant does not induce wilting in *Cmm*101; gene induces wilting when cloned into *Cmm*100 (Jahr et al., 2000)	Virulence
celB*	Other	No				
Xylanases						
xysA	Other	Yes				
xysB	Other	No	Early††			
Pectinases						
pgaA	Other	Yes	192§	8, 16‡	Mutant has reduced incidence of foliar blisters (Chalupowicz et al., 2017)	Virulence (foliar)
pelA1	PAI		192§			
pelA2	PAI					
Endoglucanases						
endX/Y	Other			8, 16‡	Mutant has reduced incidence of foliar blisters (Chalupowicz et al., 2017)	Virulence (foliar)

Table 2 *Continued*

Gene	Location	Expression in minimal medium†	Time of expression (hpi) Systemic infection	Foliar infection	Results from genetic studies	Suspected role
Transcriptional regulators						
vatr1	Other				Reduced wilting symptoms during systemic infection (Chalupowicz et al., 2017; Savidor et al., 2014)	Virulence
vatr2	Other		24, 48, 72‡	8, 16‡	Reduced incidence of foliar blisters, less wilting (Chalupowicz et al., 2017)	Virulence
wcoP			240‡‡			
Other						
gmdA	Other	No				
manB		No				
perF				8, 16, 24, 48‡	Reduced incidence of foliar blisters (Chalupowicz et al., 2017)	Virulence (foliar)
srtA					Reduced incidence of foliar blisters (Chalupowicz et al., 2017)	Virulence (foliar)
tomA	PAI					

*Presumed pseudogene.

†Transcript levels significantly greater than in medium supplemented with plant tissue homogenate for at least one time point (Flügel et al., 2012).

‡Transcript levels $\geq$ four-fold relative to that at time zero (Chalupowicz et al., 2017).

§Protein detected *in planta* at 8 days post-infection, and significantly up-regulated in medium supplemented with xylem sap vs. rich defined medium (Savidor et al., 2012).

¶Transcript levels significantly greater than in minimal medium (Chalupowicz et al., 2010).

**Proteomic analysis could not differentiate between ppaB1 and ppaB2, which only differ by one amino acid (Savidor et al., 2012).

††Transcript levels significantly greater in xylem mimicking medium vs. minimal medium (Hiery et al., 2013).

‡‡Transcript levels significantly greater at 10 days post-infection vs. in minimal medium (Flügel et al., 2012).

increased wilting symptoms when infected with *Cmm*, compared with wild-type plants (Balaji *et al*., 2008). Together, these results suggest that ethylene plays a role in defence against *Cmm* pathogenesis, and that ChpC is involved in suppression of this host defence. Reduced disease symptoms during infection with the Δ*chpC* mutant strain may be caused in part by impaired suppression of ACO1 expression by the plant host, and therefore impaired suppression of the host's defence response (Chalupowicz *et al*., 2017).

In contrast with Δ*chpC*, mutation of *chpG* in the *Cmm*101 strain has no effect on either virulence or colonization in tomato (Stork *et al*., 2008), but abolishes an apparent hypersensitive response (HR) in non-host plants (Lu *et al*., 2015; Stork *et al*., 2008). HR is a plant-induced death of cells surrounding an infection, which can prevent the spread of microbial pathogens. *Cmm* causes localized necrosis consistent with HR when infiltrated into the non-host plants tobacco and *Mirabilis jalapa*. Evidence suggesting that the observed necrosis is caused by HR rather than toxicity include a distribution of *Cmm* that is limited to the infiltrated area in the non-host plants, and the absence of necrosis when these plants are simultaneously infiltrated with inhibitors of plant metabolism (Alarcón *et al*., 1998).

In the Δ*chpG* mutant of *Cmm*101, the abolished HR could be rescued by complementation with intact *chpG*, but not with *pat-1*, *phpA* or *phpB*, indicating that the pCM2-encoded proteases do not contribute to HR. Introduction of *chpG* (but not *chpC*) into tobacco (*Nicotiana tabacum*) leaves via *Agrobacterium tumefaciens* infiltration leads to HR, which demonstrates that *chpG* is sufficient to induce this response. The study authors hypothesized that ChpG can cleave a plant-derived target, leading to conformational changes in the target, which then activate an extracellular leucine-rich repeat (eLRR) domain-containing R protein (Lu *et al*., 2015). Ultimately, this approach might be helpful to identify the substrate(s) of ChpG through testing of the cognate receptor protein in *N. tabacum*.

The genes *chpA*, *chpB* and *chpD* are believed to be pseudogenes as they have frame shifts and/or in-frame stop codons (Stork *et al*., 2008), although *chpA* is known to produce transcripts (Flügel *et al*., 2012).

Ppa family. Chymotrypsin-like serine proteases belonging to the Ppa family are encoded by 11 genes: six on the PAI (*ppaA*–*ppaE*, including *ppaB1* and *ppaB2*), four at a pair of other chromosomal locations (*ppaF*–*ppaI*) and one on the pCM1 plasmid (*ppaJ*; Gartemann *et al*., 2008). Like the *pat-1* family genes, many of these (*ppaA*, *ppaB1*, *ppaB2*, *ppaC*, *ppaD*, *ppaH* and *ppaJ*, but not *ppaE*, *ppaF*, *ppaG* and *ppaI*) produce up-regulated transcripts in minimal medium compared with medium supplemented with

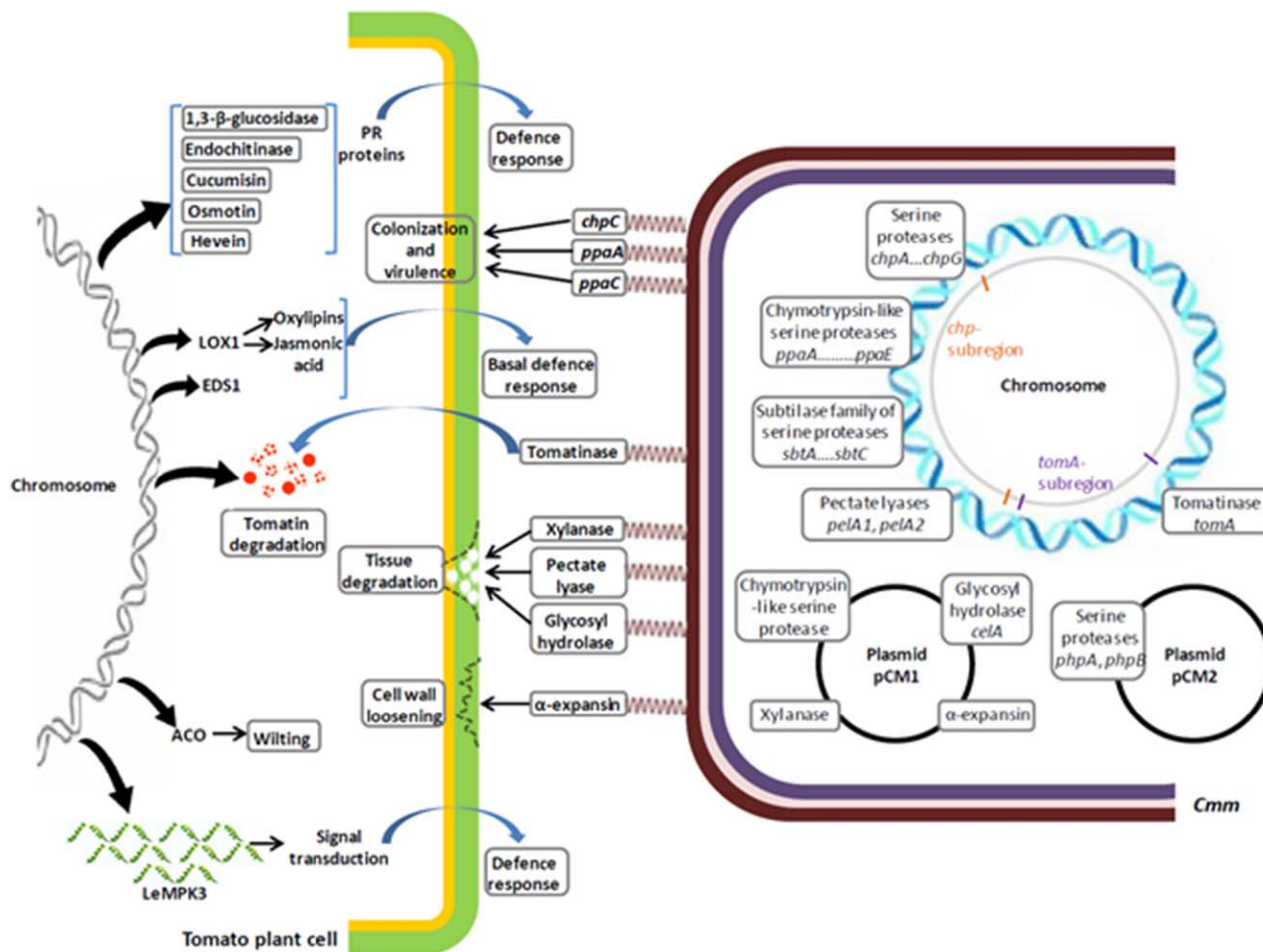

Fig. 2 Schematic diagram showing putative roles of select pathogen and host genes during *Clavibacter michiganensis* ssp. *michiganensis* (*Cmm*) infection.

plant tissue homogenate (Flügel *et al.*, 2012). In addition, the expression of *ppaB1*, *ppaB2*, *ppaC*, *ppaD*, *ppaG*, *ppaH* and *ppaI* is detected in systemically infected plants by proteomic analysis (Savidor *et al.*, 2012). Mutation of *ppaA* or *ppaC* results in lower severity of symptoms in infected plants, although the results are not statistically significant (Chalupowicz *et al.*, 2017).

Subtilase proteases. The third group of secreted serine proteases is the subtilase proteases. Of these, *sbtA* is encoded by the PAI and *sbtB* and *sbtC* are encoded elsewhere on the chromosome (Gartemann *et al.*, 2008). Intriguingly, they have high similarity to the SBT1, SBT2 and P69 subtilases of tomato (Gartemann *et al.*, 2008; Janzik *et al.*, 2000; Jorda *et al.*, 1999). Although none of the *sbt* genes are expressed in minimal medium (Flügel *et al.*, 2012), they are each detected at the transcript and/or protein level in foliar or systemically infected plants (Chalupowicz *et al.*, 2017; Hiery *et al.*, 2013; Savidor *et al.*, 2012), suggesting that they play a role after perception of the host. Mutation of each *sbt* results in lower severity of leaf blisters during foliar infection, but only mutation of *sbtA* significantly reduces the incidence of symptoms during both foliar and systemic infection (Chalupowicz *et al.*, 2017).

Cell wall-degrading enzymes

In addition to proteases, *Cmm* is able to secrete hydrolytic carbohydrate-active enzymes (CAZymes) that are involved in metabolism and degradation of plant cell wall components including polysaccharides, oligosaccharides and glycoconjugates (Cantarel *et al.*, 2009). These enzymes include cellulases, xylanases and pectate lyases, which participate in the breakdown of the plant cell wall components cellulose, xylan and pectin, respectively, to facilitate bacterial colonization and nutrient acquisition (Cantarel *et al.*, 2009). Glycome profiling of tomato plants inoculated with *Cmm*CASJ002 shows reduced arabinogalactan epitopes, and loosening of xyloglucan epitopes, which indicate severe modification of plant cell wall integrity. This modification is

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consistent with the extensive tissue damage during the later stages of *Cmm* infection (Thapa *et al.*, 2017).

Based on secretome analysis, *Cmm* strains are predicted to possess more CAZymes than other pathogenic *C. michiganensis* subspecies, as well as other Gram-positive and xylem-limited bacterial phytopathogens (Thapa *et al.*, 2017). On the contrary, tomato endophytic *Clavibacter* strains have fewer total CAZymes relative to pathogenic *Cmm* (Thapa *et al.*, 2017). Overall, the high number of CAZymes in *Cmm* suggests hydrolysis potential for a greater range of polysaccharides, and may be an indication of host specificity.

Cellulases. Located on the pCM1 plasmid, *celA* encodes a protein with high similarity to the endo-β-1,4-glucanases of family A_1 cellulases (Jahr *et al.*, 2000). Its homologue on the chromosome outside the PAI, *celB*, is believed to be a pseudogene due to a truncated C-terminal domain (Gartemann *et al.*, 2008). Although one study found that mutation of *celB* reduced the severity of leaf blisters during foliar infection (Chalupowicz *et al.*, 2017), the results were not statistically significant.

celA transcripts are abundant in minimal medium (Flügel *et al.*, 2012). Its transcripts and proteins are detected during systemic infection of tomato plants (Chalupowicz *et al.*, 2010, 2017; Savidor *et al.*, 2012), whereas transcript abundance is lower during foliar infection (Chalupowicz *et al.*, 2017). Consistent with these expression patterns, *celA* is considered to be a key player for pathogenicity in systemic infection. Like *pat-1*, introduction of *celA* into *Cmm*100, a derivative of strain *Cmm*382 that lacks both plasmids, restores virulence and results in wilting of infected plants (Meletzus *et al.*, 1993). Similarly, introduction of *celA* into a plasmid-free *Cmm*CASJ002 derivative or *Cmm*CASJ001 derivative partially restores pathogenicity (Thapa *et al.*, 2017). Furthermore, disruption of *celA* in *Cmm*101 (a derivative of *Cmm*382 which contains the *celA*-bearing pCM1, but not pCM2) abolishes wilting during infection. These results demonstrate that *celA* is a major pathogenicity gene on pCM1, and is critical to bacterial canker disease, although other pCM1 genes may affect colonization or the utilization of nutrients (Jahr *et al.*, 2000).

The 78-kDa CelA (Jahr *et al.*, 2000) is predicted to be a secreted protein, and is amongst 13 core secreted proteins present in all tomato-pathogenic *Cmm* strains (Thapa *et al.*, 2017). It consists of three domains: an N-terminal catalytic domain, a cellulose binding domain and a C-terminal domain with homology to α-expansin from plants. Removal of any one of these domains abolishes wilt induction, indicating that all domains are required for CelA-associated pathogenicity (Jahr *et al.*, 2000). The C-terminal α-expansin domain is probably involved in non-enzymatic loosening of the plant cell wall (Georgelis *et al.*, 2014) by removing hydrogen bonds between carbohydrate polymers (Cosgrove, 1998), which may improve access to cellulose for hydrolysis. An additional α-expansin located on the chromosome (Gartemann *et al.*, 2008) may also participate in pathogenesis.

Two endoglucanases encoded by the *endX/Y* gene may also participate in cellulose degradation. Transcripts from this gene are abundant during foliar infection of tomato, and mutation of *endX/Y* significantly decreases the incidence of leaf blisters during foliar infection, whereas symptoms during systemic infection are largely unaltered (Chalupowicz *et al.*, 2017). Two endoglucanases are also amongst 13 core secreted proteins present in all tomato-pathogenic *Cmm* (Thapa *et al.*, 2017).

Xylanases. Two β-1,4-xylanase encoding genes, *xysA* and *xysB*, are located on the chromosome outside of the PAI. Transcripts of *xysA* are significantly up-regulated in minimal medium relative to xylem-mimicking medium (Hiery *et al.*, 2013), and transcripts of both *xysA* and *xysB* are detected at low levels in systemically infected plants (Chalupowicz *et al.*, 2010).

Pectinases. The *Cmm* genome encodes a putative polygalacturonase, which depolymerizes pectin, and two pectate lyases which act on pectate, a product of pectin degradation in plants.

Transcripts from the polygalacturonase (*pga*), located on the chromosome outside the *chp*/*tomA* PAI, are up-regulated during the growth of *Cmm*382 in minimal medium (Flügel *et al.*, 2012) and during the early stages of foliar infection (Chalupowicz *et al.*, 2017). The protein is also detected during systemic infection, and is significantly up-regulated in medium supplemented with xylem sap vs. rich defined medium (Savidor *et al.*, 2012). Mutation of *pga* results in a significantly reduced incidence of leaf blisters during foliar infection (Chalupowicz *et al.*, 2017).

The pectate lyases (PelA1 and PelA2) are encoded on the *chp*/*tomA* PAI of *Cmm* and share 90%–92% identity (Strider, 1969; Thapa *et al.*, 2017). During systemic infection, transcripts of *pelA1* are abundant during the early stages of systemic infection (Chalupowicz *et al.*, 2010), and the protein is present *in planta*. It is also significantly up-regulated in medium supplemented with xylem sap compared with rich defined medium (Savidor *et al.*, 2012). In minimal medium, the transcript abundance of *pelA1* is greater than that of *pelA2* in strains *Cmm*382, *Cmm*CASJ002 and *Cmm*CA00002. Perhaps because of its higher expression levels, deletion of *pelA1* from *Cmm*CASJ002 leads to a reduction in wilting symptoms relative to the wild-type, whereas deletion of *pelA2* does not affect symptoms. Neither deletion affects bacterial titres *in planta* (Thapa *et al.*, 2017).

Other proteins

Other proteins found to play a role in pathogenesis include perforin and sortase. Mutation of genes for perforin (*perF*) or sortase (*srtA*) results in a significantly lower incidence of symptoms during foliar infection. The incidence and severity of systemic infection also appear to be lower in the mutants, although results in this case were not significant (Chalupowicz *et al.*, 2017).

Transcriptional regulators

Plasmid-encoded factors

Because xylanase activity is not detected in *Cmm* strains that lack pCM1, it was previously thought that the xylanase genes resided on this plasmid (Meletzus *et al.*, 1993). However, it is now known that the genes *xysA* and *xysB* are located on the chromosome, suggesting a potential role of plasmid-borne transcriptional regulators. In fact, transcripts of *xysA* are present, but significantly reduced, in *Cmm*100, which lacks plasmids, whereas their levels are not significantly altered in *Cmm*27, which lacks the PAI (both *xysA* and *xysB* are located outside of the PAI). In contrast, *xysB* transcript levels appear to be altered (up- or down-regulated at various time points) in both *Cmm*100 and *Cmm*27 (Chalupowicz *et al.*, 2010).

Other chromosomal genes whose expression is altered by the removal of plasmids include those encoding the serine proteases ChpC and PpaA, and the cell wall-degrading enzymes CelB and PelA1. Transcripts of both *chpC* and *ppaA* are significantly reduced in *Cmm* strains that lack either or both of the plasmids (*Cmm*100, *Cmm*101 and *Cmm*102). Because this reduction in expression does not appear to be additive (Chalupowicz *et al.*, 2010), transcriptional regulators on the two plasmids may participate at different positions in the same hierarchical pathway(s) that ultimately activates the transcription of *chpC* and *ppaA*.

Transcripts of *celB* and *pelA1* are up-regulated in *Cmm*100, suggesting that at least one plasmid participates in the transcriptional repression of these genes. The cellulase-encoding *celB* is also up-regulated in *Cmm*102, but not *Cmm*101 or *Cmm*27 (Chalupowicz *et al.*, 2010). These results indicate that the plasmid pCM1 is involved in the transcriptional repression of *celB*, whereas genes located on pCM2 and on the PAI are not involved.

PAI-encoded factors

As mentioned above, *xysB* transcript levels appear to be altered not only in *Cmm*100, but also in *Cmm*27, which lacks the *chp*/*tomA* PAI (Chalupowicz *et al.*, 2010). Transcript levels of *celA* (located on pCM1) and *pat-1* (located on pCM2) are also significantly reduced in *Cmm*27 (Chalupowicz *et al.*, 2017). Together, these results indicate that the PAI may encode transcriptional regulators that contribute to the expression of both chromosomal (*xysB*) and plasmid-encoded (*celA* and *pat-1*) virulence genes.

Vatr1 and Vatr2

Chromosomal genes outside of the *chp*/*tomA* PAI also play a role in the regulation of *Cmm* virulence factors. The mutation of *vatr1*, a member of the TetR family of transcriptional repressors, results in significantly higher levels of transcripts of 26 genes, including *xysB* and the subtilase protease *sbtB*, whereas the mutation of *vatr2* from the GntR family of transcriptional regulators (members of which can be repressors or activators) significantly increases the expression of 16 genes (Savidor *et al.*, 2014).

Both *vatr1* and *vatr2* also participate in positive regulatory pathways for virulence factors. Mutation analyses show that *vatr1* positively regulates 34 genes and *vatr2* positively regulates 36 genes, with 25 of these commonly regulated by both *vatr1* and *vatr2*. Amongst the genes whose transcripts are down-regulated in response to these mutations are *pat-1*, *sbtC* and *celA* for Δ*vatr1* mutants, and *pat-1* and *phpA* for Δ*vatr2* mutants (Savidor *et al.*, 2014).

Some of these regulated genes (including *pat-1*, *phpA* and *celA*) are located on the pCM1 and pCM2 plasmids. This, together with the findings that plasmids affect the regulation of chromosomal virulence genes (above), highlights the interdependence between chromosomal and plasmid genes in virulence expression. Plasmid and chromosomal genes may share *cis* regulatory elements that are affected by the same *trans* factors, and may cooperate in regulatory pathways.

Indeed, both *vatr1* and *vatr2* regulate other transcriptional regulators, indicating that they occupy high positions in the hierarchy of virulence gene regulation, and *vatr1* is a positive regulator of *vatr2* (Savidor *et al.*, 2014). All 10 of the *vatr*-regulated genes identified as transcription factors are located on the chromosome (and outside of the *chp*/*tomA* PAI).

Plants infected with the *vatr* mutants show a significant reduction in wilting symptoms during systemic infection (Chalupowicz *et al.*, 2017; Savidor *et al.*, 2014), and this reduction is much more pronounced with Δ*vatr2* mutants than Δ*vatr1* mutants (Savidor *et al.*, 2014). Mutation of *vatr2* also leads to a significant reduction in the incidence of leaf blisters during foliar infection (Chalupowicz *et al.*, 2017). Given that *vatr1* is a positive regulator of *vatr2*, the greater reduction in systemic virulence observed with the Δ*vatr2* mutant is probably the result of complete loss of its expression, compared with partial loss of *vatr2* expression in the Δ*vatr1* mutant. Neither mutation (Δ*vatr1* or Δ*vatr2*) affects the growth of *Cmm* in rich medium, demonstrating that their effect on virulence is a result of their effects on virulence genes and not on general housekeeping processes (Savidor *et al.*, 2014). Consistent with their role in virulence, plants infected with Δ*vatr1* or Δ*vatr2* mutants of *Cmm* produce significantly reduced levels of ACO and ethylene vs. plants infected with wild-type *Cmm* (Savidor *et al.*, 2014).

Host responses to infection

A number of tomato genes that are involved in defence against pathogenesis are up-regulated during *Cmm* infection, including genes involved in the production and scavenging of free oxygen radicals, enhanced protein turnover and hormone (including ethylene) synthesis and response (Balaji *et al.*, 2008).

To counteract pathogen invasion, plants can initiate signal transduction cascades involving various phosphatases and kinases (Balaji *et al.*, 2008; Beckers *et al.*, 2009; Mayrose *et al.*, 2004). In addition to known phosphatases and kinases, two phospholipase

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D signal-transducing proteins of tomato were up-regulated during *Cmm* infection (Savidor *et al*., 2012).

To combat pathogen infection, host plants activate their basal defence responses via pathogenesis-related (PR) proteins on recognition of extracellular pathogen-associated molecular patterns (PAMPs) (Nurnberger *et al*., 2004; Zipfel and Felix, 2005). Generally, these PR proteins have antimicrobial properties and are involved in cellular activities, such as defence signalling, cell wall hydrolysis, production of reactive oxygen species, contact toxicity and alkalinization of the medium (Asai *et al*., 2002; van Loon *et al*., 2006). The proteome of *Cmm*-infected tomato reveals a cluster of differentially expressed PR proteins compared with mock-infected controls, including 1,3-β-glucosidase, endochitinase, cucumisin-like serine protease, and osmotin- and hevein-like proteins (Savidor *et al*., 2012).In addition, several proteins related to specific plant defence responses are induced in the infected plant (Savidor *et al*., 2012): lipoxygenase-1 (LOX1), which may be involved in the synthesis of oxylipins or jasmonic acid (Bohland *et al*., 1997; Gardner, 1991; Melan *et al*., 1993; Vick and Zimmerman, 1983); enhanced disease susceptibility 1 (EDS1), which is crucial for the basal defence response against pathogens (Parker *et al*., 1996; Wiermer *et al*., 2005), and proteins similar to *Phytophthora*-inhibited protease 1 (PIP1) and PepEST, which are involved in the response to potential virulence factors (Ko *et al*., 2005; Tian *et al*., 2007).

Conversely, various tomato proteins are suppressed in *Cmm*-infected stems, including developmentally regulated plasma membrane polypeptide (DREPP), threonine or serine kinase for signal transduction, and four secreted class III peroxidases (Savidor *et al*., 2012). Class III peroxidases are produced during the course of lignin production, wound healing and defence, and their expression is normally up-regulated in plants on bacterial, fungal or viral infection (Harrison *et al*., 1995; Hiraga *et al*., 2001; van Loon and Geelen, 1971; Reimers *et al*., 1992). The down-regulation of these plant proteins in *Cmm*-infected tomato (Savidor *et al*., 2012) suggests that *Cmm* interferes with this plant defence response.

Overall, research related to the response of tomato towards *Cmm* infection and the mechanisms associated with symptom development is still in progress. The availability of the completed tomato genome sequence (Tomato Genome Consortium, 2012) will enable further investigations of gene functions and new strategies for disease prevention.

CONTROL OF *CMM*

Four subspecies of *C. michiganensis*, including *Cmm*, are classified as quarantine organisms by the European and Mediterranean Plant Protection Organization (EPPO) because of the serious economic threat that they pose (EPPO, 2017). Because dissemination occurs through the use of contaminated seeds and infected transplants, as well as through *Cmm*-infested soil, equipment and tools, prevention of harmful cultural practices is extremely important. Reducing plant stress by maintaining an optimum population, nutrition, weed management and fertility will also make plants less likely to become diseased.

Because of its prominent impact, research has been undertaken to develop additional efficient tools for the detection and management of *Cmm* (Fig. 3). Various reliable and sensitive assays for *Cmm* diagnosis have been discussed in earlier reviews (de León *et al*., 2011; Sen *et al*., 2015). Long-distance transmission through infected seeds means that seed testing is essential. However, although several bioassays are being developed and employed for the detection of *Cmm* in seeds, they are mostly used as presumptive tests because of their imprecise nature. Thus, the generation of a fast, sensitive and cost-efficient detection method is crucial to expedite the diagnosis at an early stage.

Chemical control of *Cmm*

Several chemical treatments for seeds, plants or soil have been studied for *Cmm* control. The most popular of these include the copper compounds, copper hydroxide and copper sulfate, and bactericides such as streptomycin, mancozeb and their combinations (Hausbeck *et al*., 2000; Kasselaki *et al*., 2011; de León *et al*., 2008; Werner *et al*., 2002). Although these antimicrobial compounds result in efficient reduction of bacterial titres, they are ultimately inadequate to protect the plant, and some are phytotoxic or promote resistance (de León *et al*., 2008; Yang *et al*., 2002). Other phytosanitary methods, such as seed treatments with acidified nitrite or 1% hydrochloric acid, and soil treatment with formaldehyde, reduce both the bacterial titre and symptom development, but are only partially effective against *Cmm* (Kasselaki *et al*., 2011; Pradhanang and Colier, 2009; Sharma and Kumar, 2000). A study using thermotherapy (hot water) for seed disinfection found that temperatures of 48 and 52 °C were effective for some seed cultivars, whereas higher temperatures (56 and 60 °C) were detrimental to seed germination and vigour (Divsalar *et al*., 2014). Therefore, the application of this eco-friendly approach will require the identification of the optimum temperature and time for particular seed varieties.

Other less conventional antimicrobials are also being studied for use against *Cmm*. For example, the hexapeptide KCM21 elicits strong bactericidal activity against *Cmm* by rupturing the bacterial cells (Choi *et al*., 2014). Twelve potent small molecule inhibitors of *Cmm* have also been found, which are various piperidines, benzimidazoles, phenols, phenoxy isopropanolamines and pyrrolidones (Xu *et al*., 2015). Using single-step and sequential passage resistance assays, no resistant *Cmm* colonies were observed following incubation at lethal and sublethal concentrations of each compound (Xu *et al*., 2015), suggesting that resistance to these compounds is not likely to be an immediate problem. However, the application of various agricultural chemicals or antibiotics faces various challenges,

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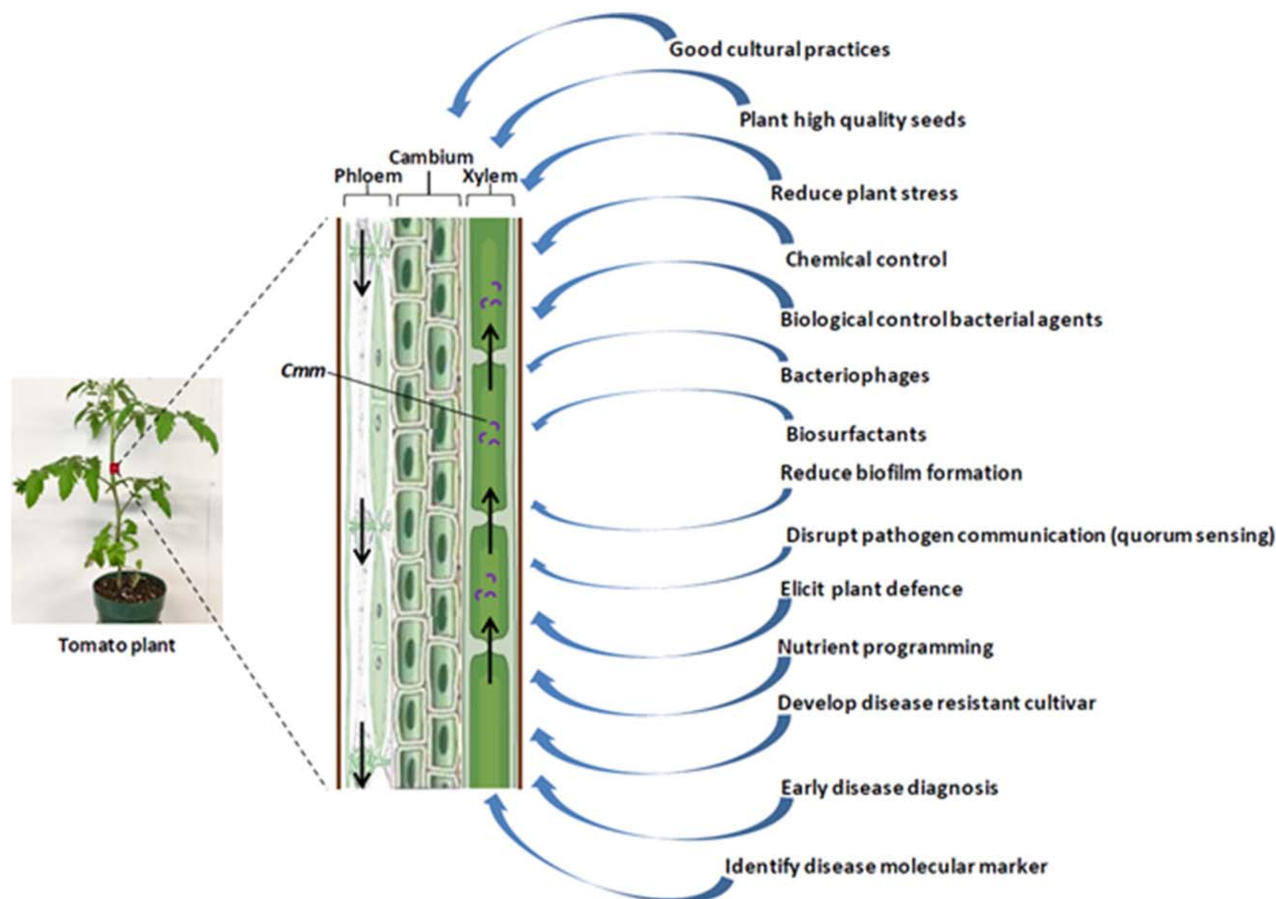

Fig. 3 Schematic diagram summarizing the strategies to manage bacterial canker disease of tomato.

including the emergence of resistant pathogens, environmental health concerns and regulatory constraints. The detailed characterization of these compounds is therefore crucial.

Plant-derived organic substances, such as extracts and essential oils, also have potential for the control of *Cmm*. Fragarin, purified from the soluble fraction of strawberry leaves, quickly inhibits the growth of *C. michiganensis* ssp. *sepedonicus* (ring rot disease of potato) via dissipation of the membrane potential (Filippone *et al*., 2001). Essential oils of thyme, oregano, dictamnus and marjoram are capable of inhibiting *Cmm* growth *in vitro* (Daferera *et al*., 2003). Related to oregano, *Origanum onites* L. provides essential oil, extracts and pure metabolites (such as carvacrol) which exhibit potent antibacterial activity against *Cmm*, and substantially decrease symptom development without affecting the germination or growth of tomato seedlings (Kotan *et al*., 2014). Moreover, the suppression of bacterial canker using tomato- or pepper-based composts in combination with chicken or cattle manure is quite effective (Yogev *et al*., 2009), and treatment with lysozyme, an antimicrobial enzyme produced by animals, significantly reduces the incidence of bacterial canker on glasshouse tomatoes (Utkhede and Koch, 2004).

In contrast with these biocidal agents, other chemicals can be used to enhance the plant's own defences towards *Cmm* (Soylu *et al*., 2003; Werner *et al*., 2002). For example, pretreatment of tomato seedlings with acibenzolar-*S*-methyl (ASM) significantly reduces *Cmm* growth and disease severity during the course of infection (Baysal *et al*., 2003). This ASM-mediated enhanced resistance is associated with increased activities of plant peroxidase and chitinase (Soylu *et al*., 2003). In addition, DL-β-aminobutyric acid (BABA) treatment remarkably suppresses symptom development caused by *Cmm* via enhanced peroxidase and phenylalanine ammonia-lyase activities of the host plant (Baysal *et al*., 2005). These findings suggest that plant defence activators, particularly ASM and BABA, are linked to the induction of tomato plant resistance to bacterial canker.

Biological control

Competing microorganisms or viruses can help to limit the growth of phytopathogens by producing biocidal substances, inducing the plant's own resistance mechanisms (Grady *et al*., 2016), or directly parasitizing the pathogen. A large proportion of such biological

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control agents consists of rhizobacteria, including many genera that are able to efficiently colonize plant roots and suppress the invading soil-borne pathogens (Schroth and Hancock, 1981; Weller, 1988).

Biocontrol agents having antagonistic activity towards *Cmm* have been reported, including seed and root treatments with fluorescent pseudomonads under glasshouse conditions (Amkraz *et al*., 2010; Boudyach *et al*., 2010). Seed treatment with both *Pseudomonas* and *Bacillus* strains improved the quality of tomato seeds and immensely decreased the incidence of bacterial canker in field studies (Kasselaki *et al*., 2011; Umesha, 2006).

Pseudomonads are well recognized for antibiosis, one of the key biocontrol mechanisms, whereby they can produce different antimicrobial metabolites, including phenazines, pyrrolnitrin and hydrogen cyanide (HCN), together with various degradative enzymes, for disease suppression (Haas and Defago, 2005; Weller and Thomashow, 1993). Accordingly, the reduction of canker disease development and *Cmm* population by *Pseudomonas* sp. LBUM300 is attributed to the production of the antibiotics 2,4-diacetylphloroglucinol (DAPG) and HCN (Lanteigne *et al*., 2012; Paulin *et al*., 2017).

Other than pseudomonads, microorganisms with *Cmm*-inhibiting activity under glasshouse conditions include *Streptomyces* sp. strain HL-12 (Yuan *et al*., 2009), *Bacillus subtilis*, *Trichoderma harzianum* and *Rhodosporidium diobovatum* (Utkhede and Koch, 2004).

Phage-based biological control is also being studied as a promising alternative because of its specificity, ease of preparation and inexpensive production (Jones *et al*., 2007). Bacteriophage CMP1, originally isolated from *Cmm*-infected, overwintering tomato stems (Echandi and Sun, 1973), is a member of the *Siphoviridae* family of viruses and specifically infects *Cmm*. Its genome encodes an endolysin with peptidase activity (Wittmann *et al*., 2011) which specifically lyses *Cmm*, but not any other bacteria (Wittmann *et al*., 2010). The phage itself may have potential in preventing *Cmm*, but, for more efficient control, its endolysin was produced recombinantly in tomato plants, giving them complete resistance to *Cmm* (Wittmann *et al*., 2016).

Resistant cultivars

As for more traditional genetic improvement, attempts to breed a *Cmm*-resistant tomato variety have been modest, and no commercial tomato cultivar possesses total resistance (Gleason *et al*., 1993; Liedl *et al*., 2013; Poysa, 1993). However, as mentioned above, *Cmm* may be present as an endophyte on some non-host plants (Thapa *et al*., 2017) and can elicit HR in others via the ChpG protein (Lu *et al*., 2015). Further research into the mechanisms that reduce pathogenicity in non-hosts may expedite the development of *Cmm*-resistant cultivars, either through traditional breeding or genetic engineering. In addition, resistance may be engineered through the expression of recombinant antibodies that interfere with key virulence factors (Safarnejad *et al*., 2011).

CONCLUSIONS

Bacterial canker disease caused by *Cmm* is a serious threat in the majority of tomato-growing areas, and is considered to be one of the most devastating plant diseases.

Current knowledge of *Cmm* implicates various virulence factors encoded by the plasmids, PAI and other chromosomal regions, yet, for many of these factors, their precise role in virulence remains unknown. The identification and study of these factors is crucial to understand the mechanism of pathogenicity, and to serve as a basic platform for the generation of target-specific bactericides. Similarly, an understanding of the molecular basis of host–pathogen interactions may identify key host factors for susceptibility and resistance, which may aid in the creation of new resistant tomato varieties.

ACKNOWLEDGEMENTS

This work was largely funded by Mitacs-Accelerate Fund (IT07941-Yuan_OGVG) and Ontario Greenhouse Vegetable Growers (OGVG). We also appreciate the support and fund from Agriculture and Agri-Food Canada, Growing Forward-II (project J-001332 and project J-001589) and conducted by the authors as a part of their duties. The project was also partially funded by the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant RGPIN-2015-06052 awarded to Z.-C.Y. We thank Niki Bennett very much for useful discussions. The authors declare no conflict of interest.

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