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PLANT DISEASE FEATURE

Bacterial Canker of Tomato: Revisiting a Global and Economically Damaging Seedborne Pathogen

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History and Economic Relevance of Bacterial Canker

Bacterial canker of tomato is an economically destructive disease caused by the gram-positive actinobacterium *Clavibacter michiganensis* (de León et al. 2011; Nandi et al. 2018; Sen et al. 2015). The pathogen systemically colonizes the xylem, causing unilateral wilt, marginal leaf necrosis, stem cankers, and ultimately plant death (Figs. 1, 2, 3, and 4) (Chalupowicz et al. 2012; de León et al. 2011; Sen et al. 2015). The bacterium can be splash dispersed onto the exterior of developing tomato fruit, producing bird's-eye lesions that can lead to contaminated seed (Figs. 1 and 5) (Medina-Mora et al. 2001; Peritore-Galve et al. 2020; Tancos et al. 2013). The first scientific report of bacterial canker was documented by Erwin Smith in the early 1900s (Smith 1910). This outbreak occurred near Grand Rapids, Michigan; therefore, the disease was called "the Grand Rapids tomato disease," which was hypothesized to be caused by *Bacterium michiganense* (Smith 1910). Since the first isolation in 1909, this pathogen has been detected globally, becoming an economically challenging problem for tomato growers worldwide.

Over five million hectares of land around the world produce 180 million tons of tomato fruit per year (FAO 2019). Outbreaks of bacterial canker are sporadic, and the severity can depend on how much inoculum is introduced, the tomato cultivars grown, weather conditions, and how rapidly detection occurs (de León et al. 2011; Sen et al. 2015). Bacterial canker causes severe economic losses every year, but additional global data on losses caused by this disease are needed to understand the distribution and severity of outbreaks. Few reports exist on the economic losses caused by bacterial canker in a given growing season. This is, in part, due to the difficulty in attributing yield losses solely to bacterial canker and not to other abiotic problems or diseases. The first reported outbreak of bacterial canker in 1910 caused $8,000 to $10,000 in economic losses for the grower, or $218,000 to $272,000 in today's U.S. dollars (Smith 1910). More recent bacterial canker reports estimated sporadic losses of up to $300,000 for Michigan tomato growers (Hausbeck et al. 2000). Yield losses can involve 46 to 93% plant death, and approximately 50% decreases in average fruit weight during severe epidemics (Chang et al. 1992a; Emmatty and John 1973; Poysa 1993).

Taxonomy of the *Clavibacter* Genus

The first Latin binomial names for the causal agent of bacterial canker were *Bacterium michiganense*, then shortly after, *Aplanobacter michiganense*, which described the nonmotile, rod-like bacterium isolated from tomato plants in Michigan (Bryan 1930; Smith 1910, 1914). The bacterium was later reclassified as *Corynebacterium michiganense* based on its irregular coryneform cell shape, and then to its current genus *Clavibacter* based on its unique 2,4-diaminobutyric acid content in the peptidoglycan layer (Collins and Jones 1980; Davis et al. 1984). The genus *Clavibacter* also contained pathogens now classified into the genera *Rathayibacter* and *Leifsonia*, leaving a sole species, *Clavibacter michiganensis* (Li et al. 2018; Suzuki et al. 1999; Zgurskaya et al. 1993). The species *C. michiganensis* consisted of six plant-pathogenic subspecies: *C. michiganensis* subsp. *michiganensis* (causal agent of bacterial canker of tomato), *C. michiganensis* subsp. *sepedonicus* (causal agent of ring rot of potato), *C. michiganensis* subsp. *insidiosus* (causal agent of alfalfa wilt), *C. michiganensis* subsp. *nebraskensis* (causal agent of Goss' wilt of corn), *C. michiganensis* subsp. *tessellarius* (causal agent of bacterial mosaic of wheat), and *C. michiganensis* subsp. *phaseoli* (causal agent of bacterial leaf yellowing of bean) (Table 1). All subspecies have been raised to the species level through whole genome and multilocus sequence approaches (Table 1) (Li et al. 2018). The current composition of the *Clavibacter* genus is *C. michiganensis*, *C. insidiosus*, *C. nebraskensis*, *C. sepedonicus*, *C. tessellarius*, *C. phaseoli*, and the recently classified species *C. capsici* (causal agent of bacterial canker of pepper) (Li et al. 2018; Nandi et al. 2018; Oh et al. 2016). New *Clavibacter* species including nonpathogenic

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endophytes have been proposed, highlighting the genetic and ecological differences within this genus (Osdaghi et al. 2020).

Epidemiology in the Field and in Greenhouse Production

C. michiganensis is a seed-borne pathogen that colonizes the tomato vasculature and fruit. Contaminated seeds can harbor between 100 and 1,000 colony-forming units (CFU) per seed, and the population threshold for disease induction is as low as 100 CFU per seed (de León et al. 2011; Fatmi and Schaad 1988; Hadas et al. 2005). Prior to the 1990s, tomato transplants were propagated in Georgia and Florida during late winter, and then shipped to growers in states with cooler climates such as New York, Michigan, and provinces in Canada (Hausbeck et al. 2000). Propagation facilities would sow tomato seeds into transplant beds, and the emerged seedlings would be routinely clipped by hand or with a rotary mower to ensure uniformity and increase plant vigor, thereby creating wounds that allow for entry of bacteria (Chang et al. 1991). The practice of grafting scions onto rootstocks that provide increased resistance to soilborne pathogens and improved horticultural traits has become increasingly common in both field and greenhouse production systems (de León et al. 2011). Cutting scions and rootstocks during grafting is another route through which *C. michiganensis* can be introduced; therefore, grafting tools should be sterilized routinely and thoroughly throughout the process (de León et al. 2011; Xu et al. 2010).

In 1984, a severe outbreak of bacterial canker occurred in commercial tomato fields in Ontario, Canada, from asymptomatic transplants harboring latent infections that came from a certified disease-free nursery in the southern U.S.A. (Chang et al. 1991; Gitaitis et al. 1991). Two important epidemiological lessons resulted from this outbreak. The first lesson was that low rates of seed contamination in transplant seedlings (0.05%, or five seeds per 10,000) can cause up to 60% of plants in the field to become systemically infected with *C. michiganensis* through mechanical transmission (Chang et al. 1991). The second lesson was that high populations of *C. michiganensis* can proliferate in the xylem of seedlings, but symptoms may not develop for up to 17 days post inoculation (Gitaitis et al. 1991). After the 1984 epidemic, new methods to detect the pathogen prior to symptom development were employed, and it became more prevalent for growers to propagate tomato transplants on site, preventing pathogen introduction from transplant facilities (de León et al. 2011; Gitaitis et al. 1991; Hausbeck et al. 2000).

After field transplantation, symptoms on tomatoes with latent infections can take between 35 and 42 days to develop (Chang et al. 1991). During this period, bacterial spread within the field can occur through pruning, trellising, and wind-blown rain (Carlton et al. 1998; Chang et al. 1991; Gleason et al. 1993). The pathogen can also be introduced to healthy fields through wind-blown rain, leaf litter, volunteer plants harboring the bacterium, and infested material such as trellising stakes (Chang et al. 1992a; de León et al. 2011; Gleason et al. 1991, 1993; Sen et al. 2015). A recent study reported white blister-like lesions forming on leaves colonized by *C. michiganensis*; however, the contribution of foliar colonization on plant health and fruit yield remains unexplored (Chalupowicz et al. 2017). The pathogen can survive between 7 and 26 months in infected plant debris at the soil surface (Chang et al. 1992a; Fatmi and Schaad 2002; Gleason et al. 1991). When plant debris is tilled 10 cm below the soil surface, pathogen survival is between 7 and 18 months. (Chang et al. 1992a; Fatmi and Schaad 2002; Gleason et al. 1991). Warmer temperatures significantly reduce the longevity of pathogen survival in plant debris (Fatmi and Schaad 2002). *C. michiganensis* is a poor saprophyte; without plant debris, it can only survive for 3 to 4 weeks in the soil (Basu 1970; Gleason et al. 1991).

Early developmental stages of tomato growth are the most important for disease detection and mitigation. A high proportion (72%) of plants inoculated with *C. michiganensis* between the 2- and 14-leaf stage develop wilt symptoms and ultimately die (Sharabani et al. 2013b). This number decreases to 30% of plants wilting and dying when inoculated at the 16- to 17-leaf stage, and no plant death when inoculated after the 19-leaf stage (Sharabani et al. 2013b). Severe bacterial canker symptoms developed when infected seedlings were transplanted to the field, leading to an average of 50% decrease in fruit yield and lower average fruit weight (Chang et al. 1992a).

The process of fruit infection is a critical stage where both the marketable product is affected and seed can become contaminated, perpetuating future outbreaks (Tancos et al. 2013). Pathogen colonization of fruit exteriors causes bird's-eye lesions, which are necrotic spots surrounded by a white halo (Fig. 5). Fruit are susceptible as early as two days post anthesis (dpa), and cease to be susceptible around 20 dpa, or when fruit are approximately at the mature green stage (Medina-Mora et al. 2001; Peritore-Galve et al. 2020; Tancos

Table 1. Plant pathogens in the genus *Clavibacter*

Disease	Causal agent	Economically relevant host	Symptoms	References
Bacterial canker of tomato	*C. michiganensis*	Tomato (*Solanum lycopersicum*)	Unilateral leaflet wilt Marginal leaf necrosis Leaf blisters Stem cankers Fruit bird's-eye lesions	de León et al. 2011; Nandi et al. 2018; Sen et al. 2015
Ring rot of potato	*C. sepedonicus*	Potato (*Solanum tuberosum*)	Marginal leaflet wilt Stem collapse Vascular necrosis in tuber	Bentley et al. 2008; De Boer and Slack 1984; Eichenlaub and Gartemann 2011
Goss' wilt of corn	*C. nebraskensis*	Corn (*Zea mays*)	Leaf scorching Leaf freckles Foliar wilt	Harding et al. 2018; Tambong 2017
Alfalfa wilt	*C. insidiosus*	Alfalfa (*Medicago sativa*)	Leaf yellowing Stunted growth Foliar wilt	Dey and Van Alfen 1979; Jones and McCulloch 1926; Li et al. 2018
Bacterial mosaic of wheat	*C. tessellarius*	Wheat (*Triticum aestivum*)	Leaf freckles Leaf spots	Carlson and Vidaver 1982; Li et al. 2018
Bacterial leaf yellowing of bean	*C. phaseoli*	Bean (*Phaseolus vulgaris*)	Leaf curling Brown leaf spots Leaf yellowing and chlorosis Foliar wilt	González and Trapiello 2014; Li et al. 2018
Bacterial canker of pepper	*C. capsici*	Pepper (*Capsicum annum*)	Stem cankers Leaf lesions and necrosis	Li et al. 2018; Oh et al. 2016

et al. 2013). The bacterium can spread from lesions on fruit exteriors into the developing fruit tissues, allowing the pathogen to colonize seed (Tancos et al. 2013). The reduction in marketable yield is further exacerbated by secondary pathogen infections causing rotting symptoms, and abiotic problems such as sunscald that often affect *C. michiganensis*-infected plants (Chang et al. 1992a; de León et al. 2011).

Warmer climates and inaccessibility to arable land, as well as climate-controlled environments leading to improved fruit quality, has increased the popularity of tomato greenhouse production. *C. michiganensis* can be introduced into these systems through infected plants and contaminated tools (Chang et al. 1991). Since greenhouses are less affected by external weather events, modes of secondary spread differ from what is observed in the field. To spread between plants in the greenhouse, the pathogen must be transmitted mechanically between plants by non-sterilized pruning shears and on workers' hands (Kawaguchi et al. 2010; Sharabani et al. 2013a). The bacterium is exuded through hydathodes in guttation fluid under high-humidity conditions (Carlton et al. 1998; Sharabani et al. 2013a). When dense populations of *C. michiganensis* are present in tomato guttation droplets in the greenhouse, workers risk mechanically transmitting the pathogen down a greenhouse row (Sharabani et al. 2013a). Therefore, it is recommended to sterilize equipment between plants, and to avoid working with plants during high-humidity conditions when guttation drops are present (Kawaguchi et al. 2010; Sharabani et al. 2013a).

Pathogenomics of *Clavibacter michiganensis*

C. michiganensis is in the phylum *Actinobacteria*, which comprises gram-positive bacteria with high guanine-plus-cytosine (GC) content in their genomes. The reference genome of *C. michiganensis*, NCPPB382, contains a circular 3.298 Mb chromosome with 72.6% GC content, and two circular plasmids, pCM1 (27.4 kbp; 66.5% GC) and pCM2 (70.0 kbp, 67.6% GC) (Gartemann et al. 2008). This strain requires both plasmids and a 129-kb pathogenicity

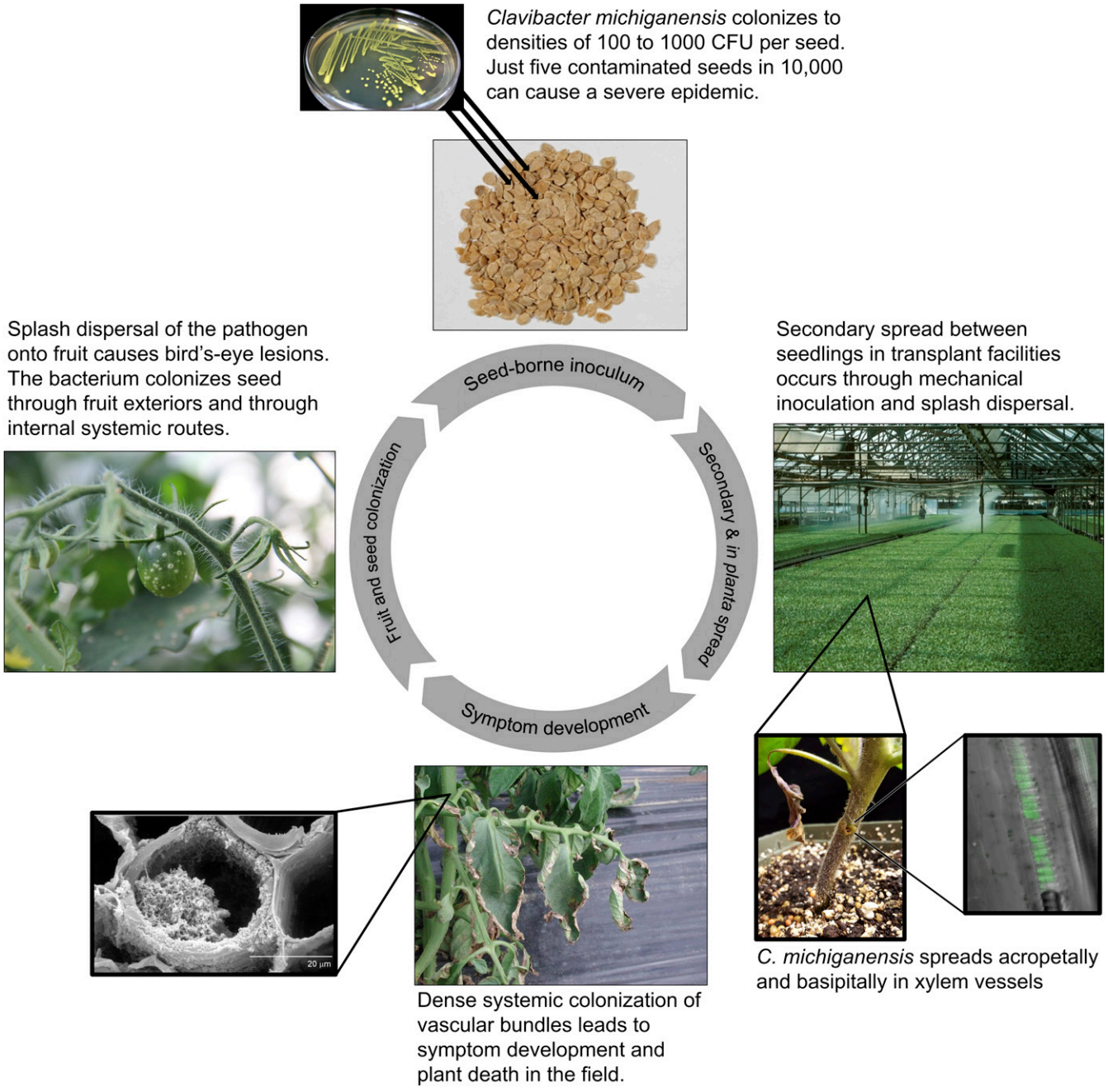

Fig. 1. The bacterial canker disease cycle.

island (PAI), termed the *chp/tomA* PAI, for full pathogenicity (Chalupowicz et al. 2010; Gartemann et al. 2008). These mobile elements encode a variety of proteases, carbohydrate-active enzymes (CAZymes), an expansin, and a tomatinase enzyme, which are putatively secreted through Sec or Tat pathways (Gartemann et al. 2008; Nandi et al. 2018; Thapa et al. 2017). Recently characterized virulent isolates have variable plasmid content, ranging from zero to three plasmids, whose genetic composition remains to be studied (Tancos et al. 2015; Thapa et al. 2017, 2020). The targets and functions of many secreted proteins remain unknown (Peritore-Galve et al. 2019), but functional genetics, genomics, and "omic" technologies have provided insights into the roles of individual genes (Table 2) (Nandi et al. 2018).

Functional genetic studies of a plasmid-cured derivative of the reference strain, Cm100, found that Cm100 could proliferate to the same density as pathogenic strains, but was impaired in systemic spread and caused no wilt symptoms (Chalupowicz et al. 2012; Meletzus et al. 1993). When one plasmid was added back at a time, colonization ability and wilt symptoms were restored, but were delayed in onset and less severe (Chalupowicz et al. 2012; Meletzus et al. 1993). These experiments concluded that genes essential for pathogenicity of the reference strain were plasmid-borne. A spontaneous mutation of NCPPB382 deleted the *chp/tomA* PAI while retaining the plasmids (Chalupowicz et al. 2010). Without the *chp/tomA* PAI, *C. michiganensis* was impaired in systemic spread, in vitro aggregation, and virulence when inoculated into the vascular

Fig. 2. Wilt, stem cankers, and leaf necrosis symptoms displayed on severely diseased tomato plants.

Fig. 3. Progression of unilateral wilt to full leaf wilt and leaf necrosis.

Fig. 4. Left, large stem canker at the site of artificial inoculation where the cotyledon has been clipped off using scissors dipped in bacterial suspension. Right, petiole canker distal from the site of inoculation on the stem.

Fig. 5. Bird's-eye lesion symptoms on artificially inoculated tomato fruit from the cultivar Ailsa Craig (top and bottom left). Bottom right, epidermal peel from tomato fruit with bird's-eye lesions.

system and onto leaf surfaces (Chalupowicz et al. 2012, 2017). These findings underscore the additive contributions of genes on the plasmids and *chp/tomA* PAI to both virulence and colonization.

The plasmid pCM2 contains a pathogenicity gene, *pat-1*, which encodes a serine protease from the chymotrypsin subfamily S1A (Table 2) (Dreier et al. 1997; Nandi et al. 2018). Targeted deletion of the *pat-1* gene in the reference strain decreased virulence significantly but did not abolish symptoms completely (Dreier et al. 1997). When expressed in nonpathogenic isolates, the *pat-1* gene caused the transformants to induce bacterial canker symptoms (Dreier et al. 1997). Recent studies have identified virulent *C. michiganensis* strains in New York and California that naturally lack the *pat-1* gene, but nonetheless are able to cause symptoms (Tancos et al. 2015; Thapa et al. 2017). Homologs of the *pat-1* gene are present on pCM2 (*phpA&B*) and the *chp/tomA* PAI (*chpA-G*) (Burger et al. 2005; Dreier et al. 1997; Gartemann et al. 2008). Seven of nine homologs have been knocked out for functional characterization in the reference strain, and only one of those seven, *chpC*, demonstrated a role in colonization, wilt, and foliar blister development (Table 2) (Chalupowicz et al. 2017; Stork et al. 2008). Eleven genes from the chymotrypsin family S1X, designated *ppaA–ppaJ*, are present in pCM1, *chp/tomA* PAI, and in other areas of the chromosome (Table 2) (Gartemann et al. 2008). Mutants of two genes, *ppaA* and *ppaC*, have been functionally characterized in the reference genome, where they had no contribution to wilt or foliar symptom development (Chalupowicz et al. 2017). The function and targets of these genes remain unknown, but infiltration of *Agrobacterium tumefaciens* expressing *chpG* was capable of causing the hypersensitive response in four *Nicotiana* species, suggesting a potential role in

Table 2. Putative virulence genes and disease severity phenotypes when each gene was mutated

Location in NCPPB382[a]	Gene	Mutant disease severity phenotype in tomato tissues[b]	Reference(s)
Chymotrypsin subfamily S1A proteases			
PAI	*chpA*	-	
PAI	*chpB*	-	
PAI	*chpC*	Reduced (wilt and blisters)	Chalupowicz et al. 2017; Stork et al. 2008
PAI	*chpD*	-	
PAI	*chpE*	None (wilt and blisters)	Chalupowicz et al. 2017
PAI	*chpF*	None (wilt and blisters)	Chalupowicz et al. 2017
PAI	*chpG*	None (wilt and blisters)	Chalupowicz et al. 2017; Stork et al. 2008
pCM2	*pat-1*	Reduced (wilt)[c]	Dreier et al. 1997; Thapa et al. 2017
pCM2	*phpA*	None (wilt)	Burger et al. 2005
pCM2	*phpB*	None (wilt)	Burger et al. 2005
Chymotrypsin-related serine proteases			
PAI	*ppaA*	None (wilt and blisters)	Chalupowicz et al. 2017
PAI	*ppaB1*	-	
PAI	*ppaB2*	-	
PAI	*ppaC*	None (wilt and blisters)	Chalupowicz et al. 2017
PAI	*ppaD*	-	
PAI	*ppaE*	-	
Chrom	*ppaF*	-	
Chrom	*ppaG*	-	
Chrom	*ppaH*	-	
Chrom	*ppaI*	-	
pCM1	*ppaJ*	-	
Subtilase proteases			
PAI	*sbtA*	Reduced (wilt and blisters)	Chalupowicz et al. 2017
Chrom	*sbtB*	None (wilt and blisters)	Chalupowicz et al. 2017
Chrom	*sbtC*	None (wilt and blisters)	Chalupowicz et al. 2017
Cellulases			
pCM1	*celA*	Avirulent (wilt)[c]	Hwang et al. 2019; Jahr et al. 2000; Peritore-Galve et al. 2020; Tancos et al. 2015, 2018; Thapa et al. 2017, 2020
Chrom	*celB*	None (wilt and blisters)[c]	Chalupowicz et al. 2017; Hwang et al. 2019
Xylanases			
Chrom	*xysA*	None (wilt and blisters)	Chalupowicz et al. 2017
Chrom	*xysB*	None (wilt and blisters)	Chalupowicz et al. 2017
Pectinases			
Chrom	*pgaA*	Reduced (blisters); none (wilt)	Chalupowicz et al. 2017
PAI	*pelA1*	Reduced (wilt)[d]	Thapa et al. 2017
PAI	*pelA2*	Reduced (wilt)[d]	Thapa et al. 2017
Endoglucanases			
Chrom	*endX/Y*	Reduced (blisters); none (wilt)	Chalupowicz et al. 2017
Expansins			
pCM1	*CmEXLX1* (CelA domain)	Reduced (wilt)[c]	Hwang et al. 2019; Jahr et al. 2000
Chrom	*expA* (*CmEXLX2*)	Increased (wilt and bird's-eye lesions)[d]	Peritore-Galve et al. 2020; Tancos et al. 2018
Others			
Chrom	*perF* (perforin)	Reduced (blisters); none (wilt)	Chalupowicz et al. 2017
Chrom	*srtA* (sortase)	Reduced (blisters); none (wilt)	Chalupowicz et al. 2017
PAI	*tomA* (tomatinase)	None (wilt)	Kaup et al. 2005

[a] PAI = *chp/tomA* pathogenicity island; Chrom = chromosomal region outside the PAI.
[b] Dashes (-) indicate that there are no reported functional genetic studies on that particular virulence gene.
[c] Genes that reduced virulence when knocked out in NCPPB382 but have contrasting results when knocked out or absent in other virulent isolates.
[d] Denotes genes whose virulence phenotype was determined in a strain other than in NCPPB382.

interactions with the host immune system (Lu et al. 2015; Nandi et al. 2018).

Plasmid pCM1 contains the pathogenicity gene *celA*, which encodes a chimeric protein consisting of cellulase, carbohydrate binding, and expansin domains (Gartemann et al. 2008; Jahr et al. 2000). Partial or full deletions of the *celA* gene in the moderately virulent Cm101 strain (Cm100 containing only pCM1) led to complete loss of virulence, establishing the essential role of each CelA domain in wilt symptom development (Table 2) (Jahr et al. 2000). Likewise, transient expression of *celA* in genetically altered and naturally nonpathogenic strains of *C. michiganensis* was capable of restoring wilt and canker symptoms, but there was no difference in colonization density with or without *celA* (Jahr et al. 2000; Thapa et al. 2017). In contrast to the essential nature of each CelA domain for full virulence of the NCPPB382 strain, a recent genetic dissection of CelA domains in strain LMG7333 found that only the cellulase and carbohydrate-binding domains were necessary for wilt induction (Hwang et al. 2019). Furthermore, mutation of the catalytic cellulase domain abolished pathogenicity, further supporting the role of cellulase activity in wilt symptom development (Hwang et al. 2019). Another chimeric cellulase-carbohydrate binding enzyme, *celB*, is encoded in the chromosome of NCPPB382 (Table 2) (Gartemann et al. 2008). However, *celB* lacks a secretion signal and is a hypothesized pseudogene due to a truncated C-terminal domain (Gartemann et al. 2008). Disruption of *celB* in NCPPB382 had no significant effect on wilt or blister symptom development (Chalupowicz et al. 2017). Functional analysis of CelB determined that it has no cellulase activity, and when modified to be secreted was not able to restore virulence in a *celA* mutant strain (Hwang et al. 2019). Despite the importance of CelA in pathogenicity of NCPPB382, pathogenic strains naturally lacking *celA* have been isolated from diseased tomato plants during bacterial canker outbreaks (Tancos et al. 2015; Thapa et al. 2020).

Expansin proteins were originally characterized in plants, where their function is to loosen xyloglucan-cellulose bonds in the cell wall in a pH-dependent manner, allowing nonhydrolytic expansion during cell growth (Cosgrove 2000, 2015). The CelA protein contains an expansin domain, CmEXLX1, which has conflicting results on its contribution to virulence (Hwang et al. 2019; Jahr et al. 2000). Another expansin, CmEXLX2, is located on the chromosome of NCPPB382 and other strains (Table 2) (Gartemann et al. 2008; Tancos et al. 2015, 2018; Thapa et al. 2017). Mutation of CmEXLX1 causes reductions of virulence or no effect on virulence (Hwang et al. 2019; Jahr et al. 2000), but disruption of CmEXLX2 in an isolate naturally lacking CelA (thus lacking CmEXLX1) led to a threefold increase in wilt symptom severity and increased bird's-eye lesion severity on fruit (Tancos et al. 2018).

The functions of CmEXLX1 and CmEXLX2 remain unknown. *Bacillus subtilis* and *Ralstonia solanacearum* expansins have weak plant cell wall extension activity in vitro and affect root binding capability (Georgelis et al. 2014; Kerff et al. 2008; Tancos et al. 2018). Localization studies of an expansin protein from the soft rot pathogen

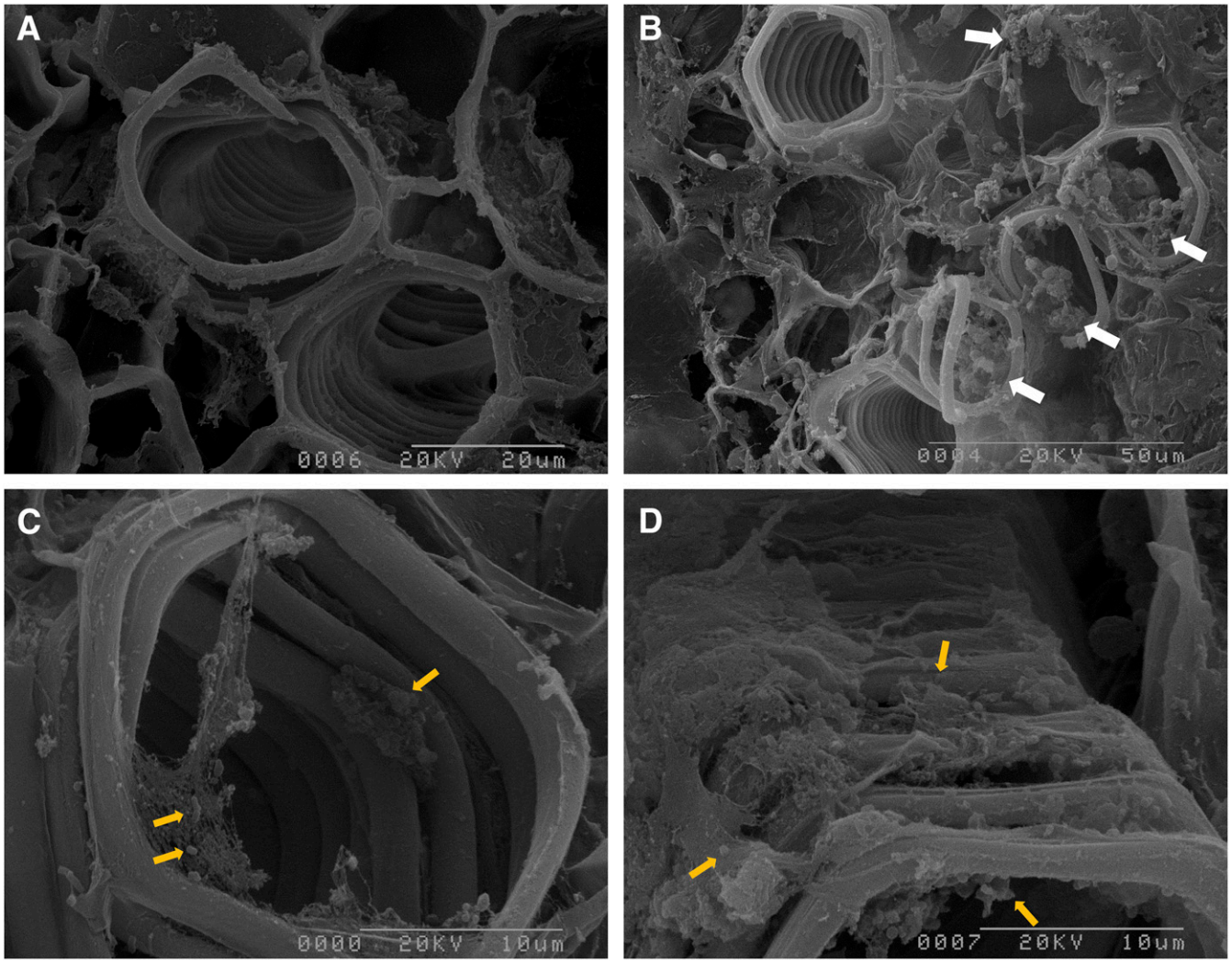

Fig. 6. Scanning electron microscopy images of xylem vessels from healthy (**A**) and infected (**B–D**) tomato plants. White arrows highlight infected vessels with bacterial aggregates. Orange arrows indicate individual bacterial cells or small aggregates within a vessel.

Pectobacterium carotovorum (PcExl1) found that PcExl1 preferentially bound celery xylem vessels and adjacent parenchyma cells (Tovar-Herrera et al. 2018). Within individual xylem vessels, PcExl1 bound to annular and helical rings of tracheary elements (Tovar-Herrera et al. 2018). The presence of PcExl1 reduced cellulase and xylanase activity in the xylem, potentially by competing for cellulose and hemicellulose binding sites (Tovar-Herrera et al. 2018). Recently, PcExl1 activity was demonstrated to induce reactive oxygen species responses from the plant, as well as increase levels of salicylic acid, jasmonic acid, and ethylene (Narváez-Barragán et al. 2020). These findings paired with the hypervirulence phenotype observed when *CmEXLX2* is knocked out suggests that CmEXLX2 may delay host responses to the pathogen by competitively binding cellulose and hemicellulose in the xylem, thereby reducing enzymatic hydrolysis by bacterial CAZymes and avoiding detection by the host through damage-associated molecular pattern-triggered immunity (Choi and Klessig 2016; Peritore-Galve et al. 2020; Tancos et al. 2018). This association is less clear with phenotypes of mutant *CmEXLX1*, which may signify different roles for both expansins, highlighting the need for future studies of these proteins.

The genome of NCPPB382 encodes an abundance of secreted CAZymes with putative xylanase, pectinase, and endoglucanase activity (Table 2) (Gartemann et al. 2008; Thapa et al. 2017). Functional characterization of xylanase-encoding genes in the reference strain identified no effect on wilt or blister symptom development (Chalupowicz et al. 2017). Mutation of a polygalacturonase-encoding gene (*pgaA*) resulted in reduced blister formation but had no effect on wilt symptoms (Chalupowicz et al. 2017). Of the two pectate lyase-encoding genes that share high sequence similarity, only one (*pelA1*) caused a significant reduction in virulence when disrupted, but there was no significant difference in colonization density between the wildtype and mutant (Thapa et al. 2017). Finally, an endoglucanase (*endX/Y*) was demonstrated to have a role in blister formation but not in wilt induction (Chalupowicz et al. 2017). Glycome profiling of stem segments infected with pathogenic and nonpathogenic *C. michiganensis* determined that the presence of pathogenic *C. michiganensis* indeed leads to the degradation of plant cell polysaccharides, consistent with the array of CAZymes encoded in the genome (Thapa et al. 2017).

Other genes have been tested for their roles in virulence. The *chp/tomA* PAI contains a secreted tomatinase enzyme, which deglycosylates the antimicrobial compound α-tomatine to tomatidine, removing its growth inhibiting properties (Table 2) (Kaup et al. 2005). Three subtilase-encoding genes are present on the *chp/tomA* PAI and other areas of the chromosome (Table 2) (Gartemann et al. 2008). Mutation of the *chp/tomA* PAI-encoded subtilase, *sbtA*, resulted in reduced blister and systemic wilt symptoms, but disruption of the other two genes had no significant effect (Chalupowicz et al. 2017). Exopolysaccharide (EPS) production is critical for colonization and virulence of the tomato vascular pathogen *R. solanacearum*; however, chemically mutated *C. michiganensis* that produced reduced amounts of EPS caused the same amount of symptoms and colonized to the same density as the wildtype (Araud-Razou et al. 1998; Bermpohl et al. 1996). Genomic analysis of NCPPB382 identified the *wcm* gene cluster as the production pathway for the major *C. michiganensis* EPS, and direct mutagenesis of those genes confirmed that EPS reduction does not affect virulence or colonization (Gartemann et al. 2008). The putative pore forming perforin protein (PerF) and cell surface anchoring sortase protein (SrtA) were necessary for full virulence during leaf blister formation, but not for wilt symptom development (Chalupowicz et al. 2017; Hendrickx et al. 2011).

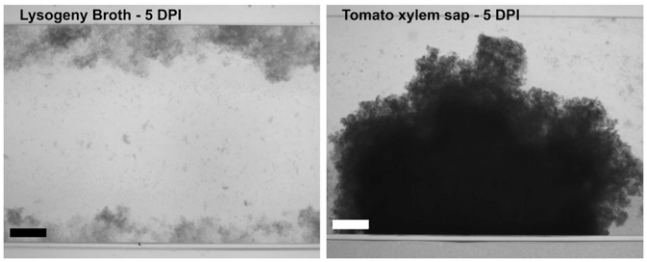

Fig. 7. Light microscopy images of *Clavibacter michiganensis* biofilm-like structures in vitro when cultured in xylem-like flow conditions in microfluidic chambers for 5 days in lysogeny broth (left) or tomato xylem sap (right). Differences in *C. michiganensis* aggregates can be seen between artificial medium and tomato xylem sap, with more aggregates forming in xylem sap at the same time point. Both black and white bars correspond to 200 μm.

Genetics of *C. michiganensis* pathogenicity and virulence have been mostly understood through studies of the reference strain NCPPB382. This strain and its derivatives are an excellent resource for investigating *C. michiganensis*–host interactions, but studies of genetic diversity have begun to describe pathogenic strains that do not require the same genetic repertoire as NCPPB382 to induce bacterial canker symptoms in tomato (Tancos et al. 2015; Thapa et al. 2017, 2020). More genome sequences and functional genetic analyses in diverse strains can provide us with a more holistic understanding of *C. michiganensis*–tomato interactions. Moreover, few functional genetic studies have studied the role of putative virulence genes in bird's-eye lesion development on inoculated tomato fruit or bacterial colonization of the phyllosphere (Peritore-Galve et al. 2020; Tancos et al. 2018). Future mutational studies of *C. michiganensis* genes should include inoculations of leaves, xylem, and fruit to exhaustively describe the contribution of individual genes to symptom development and colonization.

Host Colonization

The xylem is a fluid environment consisting of dead tracheary elements and xylem fibers, as well as living parenchyma cells. Together, these units provide plant structural stability and create conduits for passive transport of water and solutes from the roots to aerial portions of the plant. Xylem sap flows acropetally using negative tension generated by transpirational water loss and through positive pressure generated by water uptake in the roots (Venturas et al. 2017). Although the xylem is a relatively low nutrient environment, xylem sap maintains a constant flow of low concentrations of sugars, proteins, and metabolites that can be nutrient sources for pathogens (Buhtz et al. 2004; Lowe-Power et al. 2018a). Furthermore, pathogens affect xylem sap composition, which may create a more habitable environment for colonization (Dixon and Pegg 1972; Lowe-Power et al. 2018a). Therefore, with the right adaptations, pathogens can exploit the xylem for a constant stream of nutrients.

Xylem dwelling bacterial pathogens often use chemotaxis and motility systems to colonize their plant hosts. To adapt to the flow of xylem sap, the vascular bacterial pathogens *R. solanacearum* and *Xylella fastidiosa* use adhesins and EPS for aggregation and biofilm formation, and type IV pili for twitching motility (Araud-Razou et al. 1998; De La Fuente et al. 2007; Lowe-Power et al. 2018b; Meng et al. 2005; Rapicavoli et al. 2018). In contrast to these vascular bacterial pathogens, *C. michiganensis* contains no canonical pili, chemotaxis, or adhesin-encoding genes, and does not require EPS for successful vascular colonization (Bermpohl et al. 1996; Gartemann et al. 2008). Despite the absence of known aggregation and motility genes, the pathogen forms biofilm-like aggregates in xylem vessels and in vitro in the presence of xylem sap (Figs. 6 and 7) (Chalupowicz et al. 2012; Peritore-Galve et al. 2020; Tancos et al. 2018). The bacterium does not form aggregates when cultured in nutrient rich or minimal medium, but rather forms large aggregates of cells when cultured in xylem sap or media supplemented with sap, suggesting transcriptional reprogramming in the presence of a physiological cue (Fig. 7) (Chalupowicz et al. 2012; Peritore-Galve et al. 2020; Tancos et al. 2018). *C. michiganensis* NCPPB382 contains genes associated with the production and regulation of the alarmone (p)ppGpp (guanosine 3′-diphosphate, 5′-diphosphate), which plays a critical role in modifying bacterial transcription during nutrient and environmental stress conditions, termed the stringent response (Table 3) (Hauryliuk et al. 2015). These genes could play a role in inducing the expression of colonization factors and activation of virulence-associated

transcription factors (*vatr1* and *vatr2*), making them a promising target for functional analyses (Savidor et al. 2014).

C. michiganensis NCPPB382 contains adhesin-like genes, along with two operons with genes encoding tight adhesion (Tad) pili that are used for surface sensing, aggregation, and biofilm formation in *Caulobacter crescens* and *Actinobacillus actinomycetemcomitans* (Table 3) (Sangermani et al. 2019; Tomich et al. 2007). The majority of *tad* genes are not expressed in *C. michiganensis* NCPPB382 when cultured in lysogeny broth (LB) medium, which may suggest that they contribute to aggregation in the presence of xylem sap (Table 3) (Peritore-Galve et al. 2019). The xylem environment is constantly flowing with sap. Rates range as high as 8 mm^3 per second during the day and slow to 2.3 mm^3 per second at night, yet *C. michiganensis* NCPPB382 and its nonpathogenic PAI and plasmid-free derivatives are capable of spreading basipitally against the flow of xylem sap (Fig. 8) (Chalupowicz et al. 2012; Windt et al. 2006). These results suggest that *C. michiganensis* may have the capacity for active motility, and that the genes encoding this process are located in a non-PAI region of the chromosome. The recently characterized ability for Tad pili to extend and retract (Ellison et al. 2019) supports the hypothesized importance of Tad pili in colonization and motility during systemic infection.

How *C. michiganensis* causes wilt symptoms is still unknown. Studies of nonpathogenic strains have demonstrated that the pathogen can spread and colonize the vascular system to similar densities as pathogenic strains (Chalupowicz et al. 2012; Meletzus et al. 1993;

Table 3. Uncharacterized genes putatively involved in host colonization and biofilm formation

Gene name[a]	Predicted product[b]	Expressed in LB medium[c]	Predicted secretion signal[d]	Secretion supported by secretome[e]
Putative adhesins and adhesin-associated genes				
CMM_0177	ZnuA-like zinc-uptake protein/PsaA-like adhesion lipoprotein	No	SEC/SPII Lipoprotein signal peptide	N/A
CMM_0825	Putative cell surface protein containing PKD domain associated with protein-protein and protein-carbohydrate interactions	Yes	SEC signal peptide	Yes
CMM_0897	Putative adhesin or outer membrane ligand-binding protein	Yes	Tat signal peptide	Yes
CMM_2169	Putative adhesin and fibronectin-like protein	Yes	SEC signal peptide	Yes
CMM_2227	Hypothetical membrane protein associated with the type V secretory pathway adhesin AidA	Yes	No	No
CMM_2625	Hypothetical membrane protein associated with the type V secretory pathway adhesin AidA	Yes	No	No
CMM_2941	ZnuA-like zinc-uptake protein/PsaA-like adhesion lipoprotein	Yes	SEC/SPII Lipoprotein signal peptide	Yes
Putative pili and pili-associated genes				
CMM_0923	Putative Flp pilus assembly protein ATPase TadA	No	No	N/A
CMM_0924	Putative Flp pilus assembly protein TadC/ Type II secretion system F family protein	No	No	N/A
CMM_0925	Hypothetical protein containing a GspF domain associated with pilus assembly	No	No	N/A
CMM_0925A	Putative Flp pilus assembly protein TadE	No	No	N/A
CMM_0926	Putative Flp pilus assembly protein TadE	No	SEC signal peptide	N/A
CMM_1300	Putative Flp pilus assembly protein ATPase TadA	Yes	No	N/A
CMM_1301	Putative pilus assembly protein/Type II secretion system F family protein	No	No	N/A
CMM_1302	Putative Flp pilus assembly protein TadC/ Type II secretion system F family protein	Yes	No	N/A
CMM_1303	Putative membrane protein involved in pilus assembly	No	No	N/A
CMM_1304	Putative Flp pilus assembly protein TadE	Yes	No	N/A
CMM_1305	Hypothetical protein potentially involved in pilus assembly	No	No	N/A
CMM_1306	Putative Flp pilus assembly protein TadG	No	No	N/A
CMM_2295	Putative type IV pilin subunit PilE/Type II secretion system protein G	No	SEC signal peptide	N/A
CMM_2805	Putative type IV prepilin peptidase	No	Tat signal peptide	N/A
Putative stringent response-associated genes				
CMM_0135	RelA/SpoT-domain containing protein	No	No	N/A
CMM_1809	RelA/SpoT-family bifunctional (p)ppGpp synthetase/guanosine-3′, 5′-bis(diphosphate) 3′-pyrophosphohydrolase	Yes	No	N/A

[a] The locus tag assigned each coding region in the annotation of the reference genome NCPPB382.
[b] Protein products predicted from the NCPPB382 reference annotation, BLASTP, and InterPro.
[c] Refers to the presence or absence of the protein as identified in the proteome of NCPPB382 when grown to early log phase in lysogeny broth (LB) medium (Peritore-Galve et al. 2019).
[d] Secretion signal peptides were predicted using SignalP 5.0.
[e] Protein secretion was supported by peptide identification in supernatant samples of the proteome of NCPPB382 when cultured in LB medium (Peritore-Galve et al. 2019).

Thapa et al. 2017). During early stages of infection, the pathogen preferentially colonizes protoxylem vessels (Chalupowicz et al. 2012). Protoxylem are narrower, early formed vessels that support water transport during early plant growth, with wider metaxylem vessels forming after plant elongation. As wilt symptoms progress, *C. michiganensis* spreads into metaxylem and parenchyma cells potentially through the maceration of pit membranes, a strategy used by other vascular wilt pathogens but that has not been directly observed in bacterial canker (Chalupowicz et al. 2012; Peritore-Galve et al. 2020; Rapicavoli et al. 2018; Tancos et al. 2018). In tolerant wild tomatoes, *C. michiganensis* colonizes protoxylem vessels but does not spread laterally into metaxylem or parenchyma tissue (Peritore-Galve et al. 2020). Why *C. michiganensis* preferentially colonizes protoxylem is unknown, but colonizing protoxylem vessels may contribute to latency of symptom development and might serve as a route for the pathogen to spread from the seed throughout the plant (Gitaitis et al. 1991). Recent studies of xylem hydraulics determined that sap flow could occur at rates up to 15% faster in narrower vessels (i.e., protoxylem) compared with larger diameter vessels (i.e., metaxylem), which would make protoxylem vessels an even more effective conduit for systemic spread (Bouda et al. 2019).

During early wilt symptom development, typically 8 to 12 dpi, the pathogen will have spread systemically, and the host will have mounted a basal defense response (Balaji et al. 2008; Chalupowicz et al. 2012; Nandi et al. 2018; Savidor et al. 2012). This response includes an increase in pathogenesis-related proteins, production of reactive oxygen species, and increased ethylene production (Balaji et al. 2008; Coaker et al. 2004; Nandi et al. 2018; Savidor et al. 2012). Ethylene production plays an important role in wilt symptom development. When ethylene synthesis mutants and insensitive *Nr* plants were inoculated with *C. michiganensis*, the onset of symptoms was delayed by several days and wilt symptoms were less severe compared with wildtype plants (Balaji et al. 2008). Increased ethylene has been associated with the induction of vascular defenses such as tyloses and pectin gels that physically occlude pathogen spread (Pérez-Donoso et al. 2007; VanderMolen et al. 1983). These occlusions can cause hydraulic dysfunction, leading to xylem vessel cavitation and embolism, processes that are known to contribute to Fusarium wilt, Pierce's disease, and pine wilt disease symptoms (Pérez-Donoso et al. 2007; VanderMolen et al. 1983; Venturas et al. 2017). Xylem embolisms can also occur through the accumulation of hydrophobic terpenes during pine wilt disease, and through the maceration of pit membranes that connect vessels to parenchyma cells (Lowe-Power et al. 2018b; Utsuzawa et al. 2005). Interestingly, maize protoxylem vessels were more resistant to artificially induced embolisms; this relative resistance would make protoxylem vessels a safer niche than metaxylem if *C. michiganensis* indeed induces embolisms and cavitation (Hwang et al. 2016). The impacts of *C. michiganensis* on xylem hydraulics remains to be studied but may prove useful to uncovering mechanisms of unilateral wilt symptom development.

Tomato fruit colonization by *C. michiganensis* remains poorly studied compared with vascular infection. Fruit colonization is a critical step in bacterial canker epidemiology since the bacterium colonizes seed systemically and through fruit exteriors, resulting in further pathogen dispersal (Tancos et al. 2013). The hallmark of fruit infection by *C. michiganensis* are bird's-eye lesions (Fig. 5), which are white halos on the fruit epidermis surrounding a necrotic lesion. These lesions develop when *C. michiganensis* is introduced onto fruit exteriors during early developmental stages (Medina-Mora et al. 2001; Tancos et al. 2013). Fruit begin to be susceptible at 2 dpa and there is a peak susceptibility period between 5 and 7 dpa (Medina-Mora et al. 2001; Smart Lab, unpublished data). Fruit cease developing symptoms around 20 dpa, or around 3 cm diameter for the canning cultivar Ailsa Craig (Medina-Mora et al. 2001; Smart Lab, unpublished data). The diameter in which fruit cease to be susceptible to *C. michiganensis* varies by *S. lycopersicum* cultivar and *Solanum* species, but the developmental stage remains the same, suggesting a conserved change occurs in the fruit around 20 dpa that abolishes the ability for the pathogen to cause fruit symptoms (Peritore-Galve et al. 2020; Smart Lab, unpublished data).

Bacteria forming the fruit lesion spread from the epidermis into the pericarp, where they can access fruit xylem vessels for systemic spread (Fig. 9) (Tancos et al. 2013). To enter fruit xylem vessels, the bacterium must navigate through the outer epidermis, collenchyma, and parenchyma layers, which may be facilitated by a motility system. How the pathogen spreads through these tissues remains nebulous, but confocal microscopy of an eGFP-expressing isolate showed putative intracellular colonization of what appeared to be intact pericarp cells (Tancos et al. 2013). Intracellular colonization is a unique strategy rarely observed in plant pathogens, but it has been documented in two other pathogens in the phylum *Actinobacteria*: *Rhodoccocus fasciens* and *Streptomyces turgidiscabies* (Cornelis et al. 2001; Hogenhout and Loria 2008). Therefore, *C. michiganensis* may be using a combination of CAZymes and exploiting host processes to spread to fruit xylem from fruit exteriors, although the exact method remains to be studied.

Management Strategies: Breeding, Cultural Practices, and Therapeutics

Seedborne transmission paired with latent infections makes bacterial canker a difficult disease to prevent and manage. Furthermore, there are no commercially available cultivars resistant to bacterial canker and limited bactericidal products, so strategies to manage this

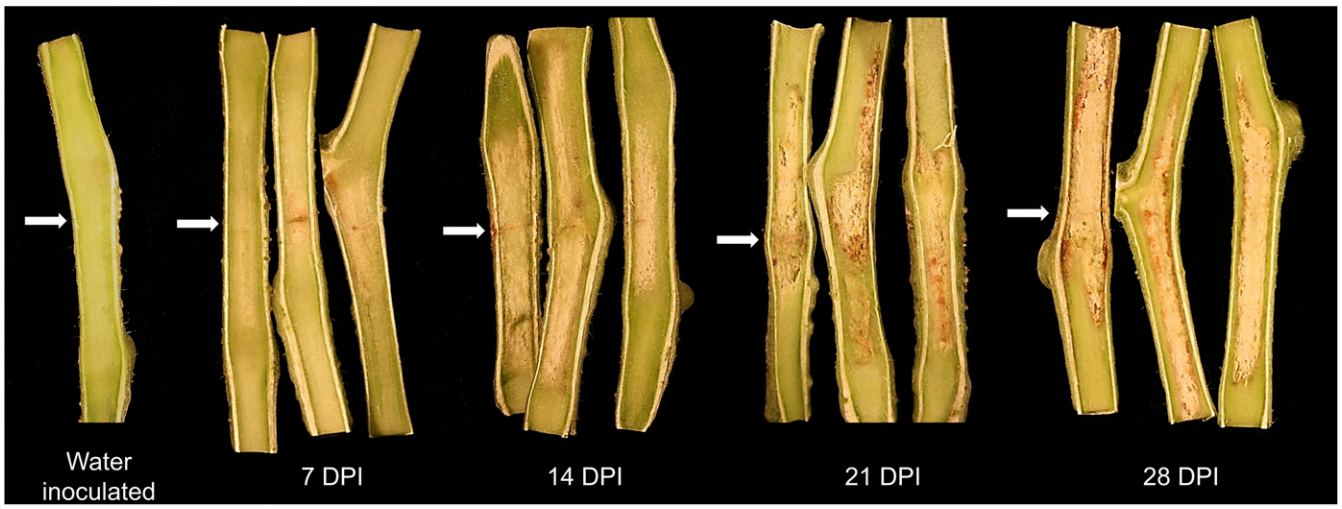

Fig. 8. Longitudinal cross sections of tomato stems with bacterial canker at 7, 14, 21, and 28 days post inoculation (DPI). White arrows indicate the position of the inoculation site.

disease must be integrated and holistic to prevent an outbreak, subsequent spread, and future epidemics (Fig. 10). Although there are currently no commercial resistant cultivars, some cultivars offer partial tolerance (Sen et al. 2015). Diverse wild tomato species are tolerant to *C. michiganensis*, developing few to no symptoms while harboring variable populations of the pathogen in the xylem (Francis et al. 2001; Kabelka et al. 2002; Lara-Ávila et al. 2012; Peritore-Galve et al. 2020; Sen et al. 2012; Sotirova et al. 1999; van Heusden et al. 1999; Vulkova and Sotirova 1993). Bacterial canker tolerance is polygenic and complex, but breeding studies have identified quantitative trait loci in *S. habrochaites* accessions that provide tolerance through changes in vascular thickness and increases in reactive oxygen species accumulation (Coaker and Francis 2004; Coaker et al. 2002, 2004; Francis et al. 2001). Transgenic plants expressing antimicrobial products such as the peptide Snakin-2, the glycan-rich extensin-like protein, and an endolysin protein from the bacteriophage CMP1 reduce bacterial populations in planta and reduce symptom severity (Balaji and Smart 2012; Wittmann et al. 2016). A more comprehensive understanding of host resistance can help identify tolerance or resistance-associated genes for incorporation into commercial cultivars through traditional breeding and transgenic approaches.

Cultural practices of using certified disease-free seed, sterilizing equipment, increasing plant spacing, and rotating crops are effective at preventing bacterial canker outbreaks (de León et al. 2011). However, sanitation and clean seed remain key pillars in reducing and preventing the dissemination of *C. michiganensis.* A European consortium known as The Good Seed and Plant Practices (GSPP, https://www.gspp.eu/) foundation was developed to prevent the occurrence of *C. michiganensis* in all steps of tomato production, ranging from seed harvesting to greenhouse management. This tomato hygiene and management system was initiated in 2009 by seed producers and growers and has since been employed by 47 international and regional companies operating in 18 countries at 107 production sites. The establishment of this international consortium that implements a GSPP standard with independent audits helps ensure that the tomato supply chain remains vigilant in preventing and managing *C. michiganensis* in our globalized economy. Moreover, it provides a common standard that can be followed to help mitigate the risks associated with tomato greenhouse production. In addition, the American Seed Trade Association publishes disease guides for commercial growers to ensure proper hygiene protocols and disease mitigation steps are properly followed (Miller and Huang 2011).

If the pathogen is present in the field, management options are limited to rogueing symptomatic plants and use of copper-based and streptomycin-containing bactericidal sprays (de León et al. 2011). Bactericides are sprayed preventatively or after symptom development in the field, and they can reduce disease severity by lowering bacterial populations (Hausbeck et al. 2000). Application of bacteriophages to control plant disease have variable results, and effective control of bacterial canker using phages has not been achieved thus far but remains a possibility through further investigation (Svircev et al. 2018). Following field outbreaks, it becomes critical to thoroughly clean all tools and equipment, deeply plow infected debris to encourage rapid decomposition, and rotate out of tomatoes for at least 1 to 3 years, depending on the environment and region (Chang et al. 1992b; Vega and Romero 2016).

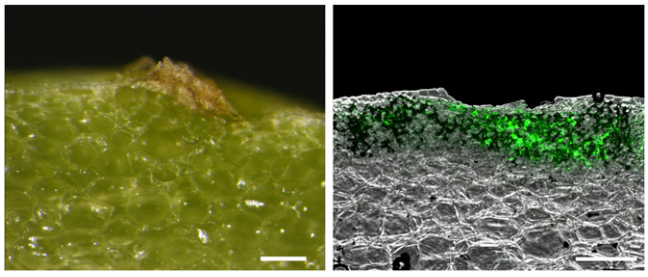

Fig. 9. Cross sections of tomato fruit lesions in the cultivar Mt. Fresh imaged using light microscopy (left) or laser scanning confocal microscopy (right). *Clavibacter michiganensis* expressing eGFP (bright green) localizes to fruit tissues surrounding the lesion. White bars correspond to 200 μm.

A variety of diagnostic methods have been employed for seed testing and detecting *C. michiganensis* in greenhouse and field environments, such as plating on selective and semiselective media, serological techniques, and DNA-based approaches (de León et al. 2011; Nandi et al. 2018; Sen et al. 2015). Increased documentation of bacteria entering a viable but nonculturable state, the characterization of nonpathogenic tomato *Clavibacter* endophytes, and the identification of *C. michiganensis* strains possessing diverse virulence gene repertoires and plasmid profiles, highlights the challenges in rapidly and correctly identifying *C. michiganensis* for tomato certification programs (de León 2011; Osdaghi et al. 2020; Thapa et al. 2017). As more tomato-associated *Clavibacter* genomes are sequenced, new DNA-based detection approaches are being developed to accurately detect *C. michiganensis* while reducing false-negative and -positive results (Thapa et al. 2020). Rapid and sensitive diagnostics for *C. michiganensis* detection in infected plant tissue and seed is constantly evolving, with new primers, tools, and approaches being developed as more cost-effective technologies and methods continually emerge.

Outlook: Prospects and Unanswered Questions in the *C. michiganensis*-Tomato Pathosystem

Bacterial canker continues to cause significant economic losses to growers worldwide, yet we are just beginning to understand how the pathogen causes symptoms in vasculature, leaf tissue, and fruit. Many biological and pathological questions remain unanswered, but recent improvements in omics technologies (i.e., genomics, transcriptomics, proteomics, and metabolomics) have the potential to uncover important biological information about the bacterium and processes at the host-pathogen interface that are critical to disease. A comprehensive understanding of both pathogen and host biology will complement ongoing research into resistance breeding and management strategies. Together, these approaches can reduce the impact of bacterial canker on tomato growers. In this section we highlight important aspects of the pathogen and host that remain unexplored and suggest the integration of new technology to aide our understanding of bacterial canker.

Leveraging New Technology for a Systems-Level Understanding of Virulence and Disease

Technological advances and reduced costs have made next generation sequencing platforms attractive tools for understanding the genetic composition of pathogenic and nonpathogenic *C. michiganensis*. Recent characterization of diverse endophytic, nonpathogenic *Clavibacter* species associated with tomato highlights the diversity and importance of horizontal gene transfer events that may lead to the reshuffling of virulence genes, plasmids, and pathogenicity islands, which presumably generates pathogenic isolates. Characterizing and investigating nonpathogenic *Clavibacter* species associated with tomato is not only important to understanding global movement and evolution but is also necessary for the development of thorough detection and management strategies to ensure clean seed production and potential plant resistance. Furthermore, a pan-genome of the *Clavibacter* genus would enable comprehensive analyses of genes that may determine host specificity as well as symptom induction in different tissues of each host. From the host perspective, a pan-genome of diverse tomato species has been generated (Gao et al. 2019). This resource can be combined with in planta experiments assessing pathogen colonization and spread, host symptom severity, anatomy, and immune responses to delineate genetic factors underlying host tolerance, resistance, and susceptibility.

Beyond the gene level, transcriptomics, proteomics, and metabolomics of different host tissues from diverse host plants can provide systems-level information about bacterial virulence factors and host responses during infection. These experiments can be carried out using pathogenic and nonpathogenic strains and can integrate genomic information from the strains and hosts used in the experiments to

discover specific pathogen and host pathways involved in colonization, symptom development, and host resistance. Ultimately, the goal would be to use these data to guide breeding and transgenic approaches for less susceptible tomato plants.

Host and Bacterial Roles in Symptom Development

C. michiganensis systemically colonizes xylem vessels, yet we still do not know how the pathogen spreads acropetally, basipetally, and between xylem vessels. Genomic analyses of the pathogen have identified putative motility genes that would assist in movement against the flow of xylem sap (Table 3), and experimental evidence has demonstrated that the pathogen forms biofilm-like structures in the presence of xylem sap, but not in artificial media (Fig. 7) (Chalupowicz et al. 2012; Gartemann et al. 2008; Peritore-Galve et al. 2020; Tancos et al. 2018). Future studies on biofilms, motility, and bacterial signaling involved in these different lifestyles could help develop therapeutics that target the bacterium in the xylem, or assist in breeding tomato lines that do not produce the cues or environmental conditions in xylem sap that support *C. michiganensis* growth and biofilm formation.

Host pathways involved in wilt induction, canker formation, and plant death remain nebulous. It is still unclear if leaflet wilt is caused by the formation of biofilm-like structures that occlude xylem sap flow, by host immune responses that are deleterious to plant health, or some combination of both. New technologies and interdisciplinary collaborations can be used to probe questions of xylem hydraulics during *C. michiganensis* infection. Understanding how water transport is affected by colonization of pathogenic and nonpathogenic *C. michiganensis*, and how these responses differ in tolerant versus susceptible cultivars may be critical to our comprehension of this disease. This line of research can be complemented by studies of different vascular morphologies implicated in host tolerance, as well as multiomics studies of host responses to understand immunity against xylem-colonizing pathogens.

Infection processes of *C. michiganensis* in tomato fruit have remained on the sideline of *Clavibacter* research. However, as the understanding of vascular infection improves, we must make sure to develop breeding or management strategies to reduce the impact of fruit infection on seed contamination and yield loss in the field. Several lines of evidence indicate that fruit are only susceptible during early periods of development (Medina-Mora et al. 2001; Tancos et al. 2013; Smart Lab, unpublished data), yet we do not know what factors are involved in immunity or lack of lesion formation at the fruit level, or how the pathogen bypasses the waxy cutin layer. Furthermore, it is unknown whether bird's-eye lesions are symptoms developed by the fruit, or visual signs of pathogen colonization and penetration into deeper fruit tissues. It is also unclear whether pathogenesis of *C. michiganensis* confers a fitness advantage over nonpathogenic *C. michiganensis* or if symptom development is simply "collateral damage" of the plant responding to a xylem inhabitant (Thapa et al. 2019). Studying seed colonization of pathogenic versus nonpathogenic isolates of *C. michiganensis* through systemic routes may uncover important information about potential fitness advantages of pathogenesis.

Function and Structures of *C. michiganensis* Virulence Factors

Many virulence factors with putative roles in host colonization and infection have been identified within the *C. michiganensis* genome through a multitude of comparative genomic studies and systematic gene knockouts (Table 2). However, in order to identify the

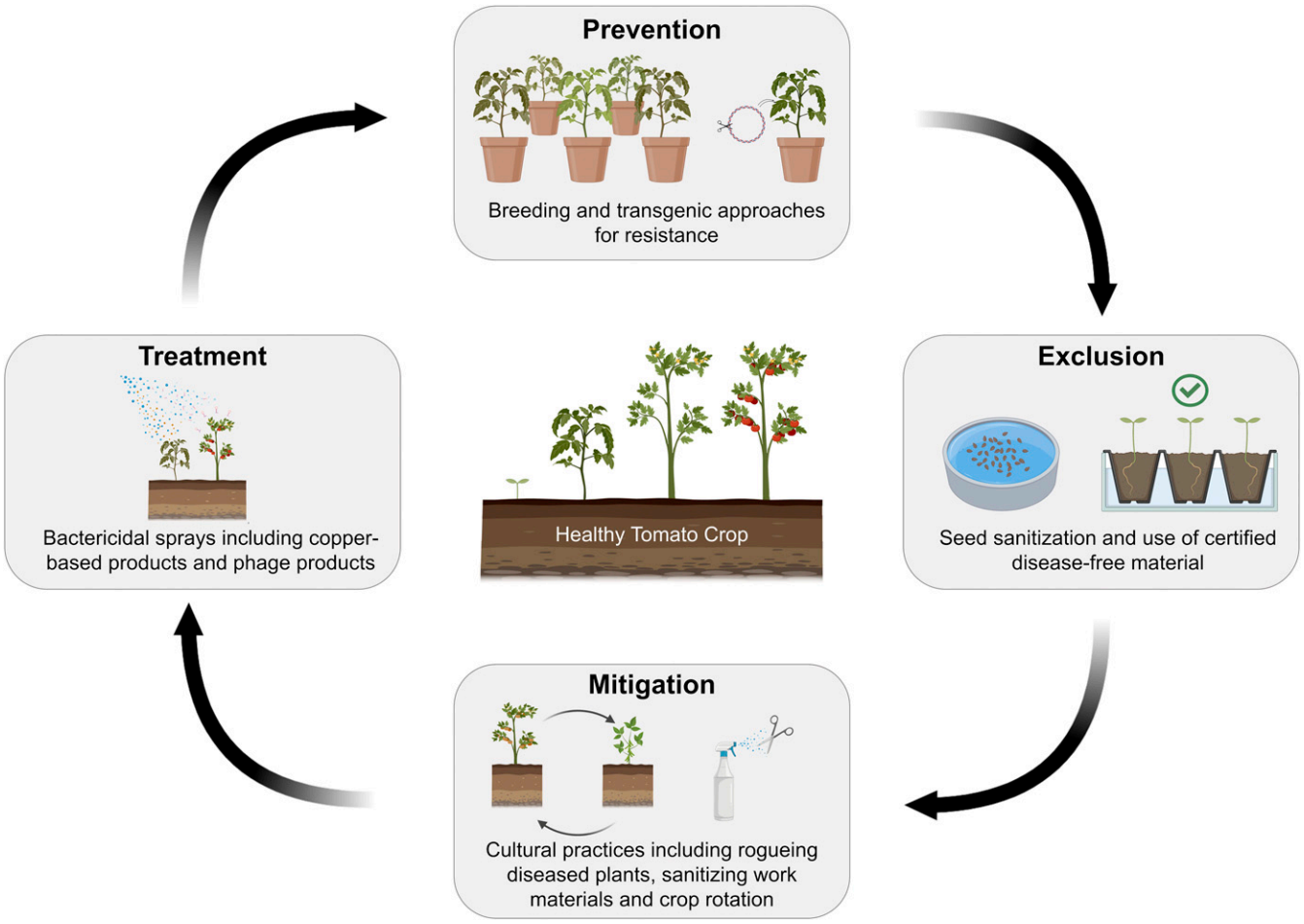

Fig. 10. Integrated approaches for prevention, management, and treatment of bacterial canker.

molecular functions of pathogenicity and virulence-related proteins and their role in host infection and colonization, future molecular studies will require multifaceted approaches including microscopy, multiomics experiments, and immunofluorescence analyses. To understand the role of individual virulence factors during infection, the *Clavibacter* field must study the effect of gene mutants on infected xylem, tomato fruit, and leaf tissue. Detailed in situ subcellular localization of *C. michiganensis* pathogenicity-related proteins, and multiomic analyses of bacterial mutants in diverse tomato hosts and their various susceptible tissues will provide unique insights into host-microbe interactions and disease development.

The functions and targets of secreted *C. michiganensis* serine proteases, subtilases, CAZymes, and expansins remain mostly unknown, as do the secretion systems used to export these proteins. Advances in structural biology using cryo-electron microscopy have enabled hypothesis-generating and hypothesis-driven experiments in bacterial systems. This technology may enable characterization of individual *C. michiganensis* virulence factors at the atomic level, which has the potential to uncover catalytic domains and structural modifications of the proteins in the presence of xylem sap. Pairing this technology with in situ localization and multiomic data can thoroughly delineate host-microbe interactions critical to colonization, systemic spread, and host responses.

The role of microbial expansins in host colonization and pathogenesis appear to be as diverse as the bacteria that possess them (Tancos et al. 2018). Pathogenic *Clavibacter* species infect a variety of host plants that include both monocots and dicots, which makes *Clavibacter* an ideal model system for future microbial expansin studies. A putative nonchimeric expansin was identified in the monocot-infecting pathogen, *C. tessellarius*, which causes bacterial mosaic of wheat. The conservation of homologous nonchimeric expansin proteins in two diverse phytopathogenic *Clavibacter* species provides a unique opportunity to explore the interactions of bacterial expansins with monocot and dicot cell walls, which may help identify binding sites and functions of these microbial proteins.

Primary cell walls of dicots and monocots vary in both structure and architecture, and vascular plants evolved diverse expansins, comprising of α-expansin, β-expansin, expansin-like A, and expansin-like B families, to modify and loosen cell walls (Cosgrove 2015). The α-expansins appear to play a dominant role in dicots, while β-expansins appear to be more prevalent in grasses and other monocots (Cosgrove 2015; Sampedro et al. 2015). However, the functions, binding sites, and molecular roles of microbial expansins in cell wall attachment may become more evident with molecular studies investigating homologous expansins from related pathogens, but diverse monocot and dicot hosts.

Finally, large-scale characterization of virulence-associated genes in diverse *C. michiganensis* strains has been impeded by the difficulty of transforming the bacterium. Current strategies for manipulating the pathogen include chemical mutagenesis (Bermpohl et al. 1996), transposon-mediated mutagenesis (Gartemann and Eichenlaub 2001; Hwang et al. 2019), and targeted gene disruption through homologous recombination (Laine et al. 1996; Tancos et al. 2018). Recent advancements have been made in CRISPR-mediated mutagenesis systems to edit the genomes of intractable bacteria (McAllister et al. 2017; Peters et al. 2019). Adapted CRISPR interference or CRISPR knockout plasmids would be an important genetic resource for the *Clavibacter* community, improving transformation efficiency, enabling studies of pooled mutant bacteria, and could be potentially used to genetically manipulate other pathogens in the family *Microbacteriaceae*.

Integration of Knowledge to Advance Prevention and Management

Although the research suggested thus far is aimed at answering basic biological and pathological questions, applied research must continue to optimize current bacterial canker control strategies in the greenhouse and field. Highly sensitive assays to detect pathogenic *C. michiganensis* in seed lots are critical to excluding the pathogen. However, once the pathogen has been introduced it takes rigorous sanitation measures and crop rotation to eliminate and prevent future outbreaks. Further research into antimicrobial development for foliar sprays and soil drenches can be aided by understanding *C. michiganensis* biology. Disease-suppressive soils may also provide conditions that can improve host immunity against this disease or outcompete the pathogen in leaf litter and soil surfaces. Finally, management strategies and therapeutics must be complemented by tomato breeding programs working toward improved resistance or tolerance against bacterial canker.

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