Bacterial Canker of Tomato: Current Knowledge of Detection, Management, Resistance, and Interactions

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Clavibacter michiganensis subsp. *michiganensis* (Smith) Davis et al. is the causal agent of bacterial canker of tomato (*Solanum lycopersicum* (Mill.)). The disease was first described in 1910 in Michigan, USA (84). *C. michiganensis* subsp. *michiganensis* (from now on called clavibacter) was initially thought to be a phloem parasite, but was later found to be a xylem-invading bacterium (15).

Clavibacter can grow at temperatures of 20 to 30°C, can survive at 50°C, and grows optimally at 25°C. Clavibacter can grow on artificial media, but from 3 to 7 days are needed for colonies to become visible on selective agar plates. The optimum pH for growth of this bacterium is between 7 and 8, but clavibacter still grows in plant xylem at pH 5 (31).

The *C. michiganensis* subsp. *michiganensis* host range comprises mainly solanaceous crops such as tomato, pepper, and eggplant. Strains of clavibacter show great variability in virulence (1) and are usually described as being hypervirulent, hypovirulent, or nonvirulent.

Molecular typing of bacterial strains based on genomic fingerprinting (rep-PCR) analysis revealed four haplotypes (57,58,74), and a combination of rep-PCR and multilocus sequence typing (MLST) analysis identified seven groups of bacterial strains (68). The genome of clavibacter strain NCPPB382 has been sequenced (43); the circular chromosome consists of 3,298 Mb and has a high GC content (72.6%). In total, 2,984 coding DNA sequences were found, 2,029 of which could be annotated. Approximately 20 *C. michiganensis* subsp. *michiganensis*-specific regions with a low GC content were found, with the largest being the Chp/tomA region. The low-GC-content regions are thought to be of foreign origin and introduced via horizontal gene transfer (43). Clavibacter harbors a high number of transporters and transcriptional regulators and is therefore very similar to soil bacteria, indicating that the

pathogen is recently evolved from the plant-associated Microbacteriaceae (43). Consistent with this hypothesis, clavibacter can survive in plant debris for up to 2 years (35).

Bacterial canker caused by *C. michiganensis* subsp. *michiganensis* is considered to be the most important bacterial disease affecting tomatoes, and yield losses can be severe. Yield losses vary with year, location, cultivar, and phenological stage of host infection; for example, in Ontario, Canada, it accounted for yield losses of up to 84% in commercial fields, whereas in artificially inoculated crops, yield losses varied from 46 to 93% (73). The economic losses can also be high: in Michigan, bacterial canker has cost individual processing tomato growers as much as \$300,000 in a single year (47). As a result of severe yield and economic losses, *C. michiganensis* subsp. *michiganensis* is a quarantine organism in the European Union and in many other countries (26).

Clavibacter lacks a type III secretion system (16), and only a few virulence factors have been experimentally determined from the many putative virulence factors. As the molecular mode of infection by clavibacter is unknown, researchers have avoided intensive work on this organism. Genetic plant mechanisms conferring resistance to clavibacter are apparently complex, and breeders have yet to develop disease-resistant cultivars.

The Pathogen

Life cycle. Infected seed and infested plant debris are the primary sources of inocula for disease outbreaks (74), and seed is the primary vector over long distances. Seed to seedling pathogen transmission rates can vary from 0.25 to 85% (88), and population densities as low as five bacterial cells per seed can result in infected seedlings (63). Even a transmission rate of 0.01% can initiate a serious epidemic under favorable conditions (18). The subsequent interplant spread (secondary infection) of clavibacter within an established crop occurs mainly via cultural practices such as pruning, clipping, contact between infected and healthy plants, rain, splashing, irrigation practices, and delivery of nutrient solutions during fertigation (14,75,102). Secondary infections occur through stomata, hydathodes, roots, and damaged tissues, including injured trichomes (59). After infection is established, clavibacter invades xylem vessels, whereby it moves systemically throughout a host. When infections occur on mature plants, they may be

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asymptomatic: yield loss was not significant when plants were infected after the 18-19 leaf stage (83). As asymptomatic plants can serve as a source of infection in the subsequent growing season, farmers ought to employ stringent precautions against many potential pathogen sources before starting a new growing season. The life and disease cycles of *Clavibacter* are depicted in Figure 1.

Bacterial movement in a plant. Understanding the colonization of a tomato plant stem by *Clavibacter* is important for the study of disease resistance mechanisms. At the start of an infection, the bacteria that enter a tomato plant are unequally distributed, and so the absence of bacteria in some plant organs or tissues does not mean a complete absence within a plant (102). Light-microscopic observations of bacteria in the petioles of susceptible plants 5 weeks following inoculation showed that the population size of bacteria was increased in some parts of the xylem tissue (72). Initially, the bacteria multiply between the spiral thickenings and then later fill the xylem lumina. When the bacteria enter the xylem vessels, there is a rapid upward and lateral spread, and this spread into adjacent xylem bundles explains the appearance of one-sided wilting, a characteristic symptom of bacterial canker. Subsequently, the bacteria begin to degrade the primary cell walls of phloem tissue, destroying xylem vessels and subsequently phloem tissues as the bacteria multiply. In contrast with the rapid movement of bacteria in the xylem, bacteria do not move freely in phloem tissue due to the structural morphology of its sieve tubes. When the roots of susceptible tomato plants were inoculated, the pattern of bacterial spread was similar to that for petiole inoculations: first, the xylem was colonized and thereafter the entire plant (100). Extracellular enzymes are thought to be responsible for the degradation of the primary walls and the middle lamella of the xylem and phloem tissues. No vessel-plugging materials, such as degraded plant products, bacterial extracellular polysaccharide material (EPS), or masses of bacterial cells, are observed in infected xylem tissue. Bacteria are able to colonize the seed coat and endosperm from within the plant or externally (89). Following germination of infected seeds, the bacteria move from the seed coat to the cotyledon, where disease development ensues (103). Our research (82) showed that although bacterial movement in the plant is spatially uneven and unpredictable at the beginning of an infection, the bacteria are able to spread throughout the plant during the later stages of disease development.

Detection. *C. michiganensis* subsp. *michiganensis* is categorized as a harmful organism by the European Union (Annex II A, section II of Directive 2000/29/EC as amended). For this pathogen, the availability of sensitive, reliable, fast, and inexpensive detection methods is indispensable for the development and employment of effective disease management strategies. Detection is also important in tracking infected plant material during disease out-

breaks. A generally accepted pathogen detection method is the dilution plating of seed or plant extracts onto a semiselective medium for *C. michiganensis* (SCM), and D₂ANX, and if needed, confirmation of suspected colonies by bioassay (51). To prevent the spread of the pathogen by seed, the International Seed Federation requires that the pathogen cannot be detected in 10,000 seeds using two selective media (50). Serological, genetic, bioassay, and dilution plating methods are currently used for detection of *Clavibacter* (Fig. 2), and the strengths and weaknesses of each detection method are discussed below.

Serological methods for detecting *Clavibacter* include enzyme-linked immunosorbent assay (ELISA), immunofluorescence (IF), immunofluorescence colony staining (IFC), and immunomagnetic bead separation (IMS). The specificity and detection level of ELISA depends on the quality of the antibodies and whether they are monoclonal or polyclonal. Monoclonal antibodies against *Clavibacter* can detect 99% of strains (55); however, cross reactions with other saprophytes have been observed using polyclonal antibodies (40). The ELISA detection limit varies between 10³ and 10⁴ CFU/ml (28), but the differences between dead and viable cells cannot be determined, and cross-reaction with other saprophytes, especially with *Clavibacter lookalikes*, can occur (40,52). ELISA is used routinely (81), and kits are commercially available. For immunofluorescence (IF) assays, individual bacterial cells are fixed to a microscope glass by heat or alcohol fixation. Fluorophore-conjugated antibodies are combined with and bind to the outer cell wall of any *C. michiganensis* subsp. *michiganensis* bacterial cells present on the glass, and the fluorescent cells are visualized using epifluorescence microscopy. The detection level of IF is 10³ CFU/ml for naturally contaminated seeds (71), and in routine testing programs, samples testing positive by IF are re-examined on selective media. Immunofluorescence colony staining (IFC) identifies immunostained target colonies and requires 3 to 5 days to complete due to the time required for colonies to form and be treated with fluorophore-labeled antibodies. Consequently, IFC detects only living bacteria and relatively large amounts of antibodies are used, making this technique expensive; however, as few as 10 CFU/ml of the pathogen can be detected (97), making it one hundred times more sensitive than IF (70). Immunomagnetic bead separation (IMS) is based on the use of immunomagnetic beads coated with specific antibodies to capture target bacterial cells. This method permits the removal of nontarget cells prior to plating on a nonselective medium and has a detection threshold of 10 CFU/ml in a heterogeneous seed mixture (27), meaning that IMS-plating is more sensitive than dilution plating on semiselective media, IF, ELISA, and PCR techniques (25). IMS can also be used in combination with PCR (IMS-PCR).

Genomic methods are based on the detection of specific nucleic acid sequences via DNA amplification by PCR. The pathogen sequence is available and the pathogenic regions of bacteria are known. DNA primers for the *ceiA* and *pat-1* virulence factors can distinguish the *C. michiganensis* subsp. *michiganensis* subspecies from other *C. michiganensis* subspecies. Although it is possible to distinguish between virulent and avirulent *C. michiganensis* subsp. *michiganensis* strains using these two virulence factors, their presence does not always correlate with the induction of symptoms (9,58,106). The PCR method has a detection level of 10² CFU/ml in a plant homogenate (28). Until now, a new detection method using primers based on the putative two-component system sensor kinase (Ptssk) sequence gave no false negatives or false positives (10). A disadvantage of PCR techniques is that they do not distinguish between dead and viable cells. Alternatively, Bio-PCR uses bacteria cultured on agar media prior to PCR and is a sensitive technique that primarily detects viable cells, and it can detect one infected seed per 10,000 seeds (45). PCR and Bio-PCR do not quantify pathogen levels, they only show whether a pathogen is present or not. Real time PCR (RT-PCR) can be used for quantification, but this technique can lead to incorrect conclusions arising from the presence of dead cells or PCR inhibitors (80). TaqMan RT-PCR has been used to quantify and differentiate *C. michiganen-*

Fig. 1. Infection cycle of *Clavibacter michiganensis* subsp. *michiganensis* in tomatoes. The primary inoculum is usually infected seeds (1), with secondary infections being caused by above- and belowground transmission of the pathogen through roots (2), leaves (3), cultural practices (4), and infected debris contamination (another primary infection source) during the next cycle of growth (5). Infected seeds begin a new cycle (6).

sis subspecies in buffer suspensions (3), but the sensitivity level was not reported. In combination with ethidium monoazide (EMA), TaqMan RT-PCR could differentiate between dead and viable cells with a detection level of 10^3 viable cells/ml (66), and in our studies we were able to detect viable bacteria with a sensitivity of 10^2 CFU/ml using an improved TaqMan probe (82).

Dilution plating onto selective media allows the quantification, isolation, and full identification of viable target pathogen cells; however, it is relatively time-consuming and laborious, requiring 5 to 14 days to obtain results. Moreover, the efficiency depends on the microbial background, as the growth of clavibacter colonies can be inhibited by the presence of other microorganisms. Clavibacter shows a great variability in growth characteristics on different selective media, so the use of more than one medium is recommended. Selective media options include semiselective medium (SCM), minimum semiselective medium (mSCM), fast semiselective medium (SCM-fast), D₂ANX, and *Corynebacterium nebraskense* selective medium (CNS). Nonselective media include yeast extract dextrose (YDS) and nutrient broth yeast (NBY) (12,20,34). Using SCM, the presence of one infected seed containing >50 bacteria can be detected in 10,000 uninfected seeds (34). Target and nontarget bacteria on tomato seeds were detected using both SCM and D₂ANX, and 95% of the colonies on both selective media were indeed *C. michiganensis* subsp. *michiganensis*, with detection levels of 1 to 10 CFU/ml (71). Recently, a new medium, BCT, was introduced, and has a better recovery rate in the presence of a high saprophytic background (41). Although the media plating technique is very sensitive, false negative results can be caused by either low levels of the target bacteria (71) or high microbial backgrounds (J. van der Wolf, unpublished results).

Bioassays are based on the inoculation of susceptible tomato plants with a pure bacterial culture, and subsequent inspection for the development of typical symptoms caused by clavibacter. Bioassays are used to distinguish clavibacter from bacteria with similar morphologies and to determine the relative virulence level (1,55); however, bioassays using seedlings require at least 21 days (40,51) before symptoms can be observed reliably.

In summary, each diagnostic method has its own advantages and disadvantages. Serological methods are relatively fast and inexpensive but risk cross-reaction (aspecificity) with other organisms (false positives). Moreover, their sensitivity is limited and can result in false negative results. Molecular techniques can also produce false positives or false negatives if PCR inhibitors are present. In culture, clavibacter can be overgrown by nontarget organisms or overestimated as a result of bacteria with similar appearance, so colony identity must be confirmed using other methods. Pure bacterial cultures are required for bioassays, and such procedures require weeks to complete. Different methods, or a combination of methods, can be used to detect and quantify clavibacter depending upon whether the samples are seeds or plants, if the purpose is detection or quantification, or if the time required is fast or slow.

The Disease

Bacterial canker. Bacterial canker of tomato is a worldwide problem (89) and is the most important bacterial disease affecting tomatoes (67). During the early stages of disease development, unilateral wilting of leaflets and leaves is common (Fig. 3C). Cankers, for which the disease is named, then develop on stems and petioles during the later stages of pathogenesis (Fig. 3F and G). Characteristic birds-eye spots develop on infected tomato fruits and

Fig. 2. Methods for the detection and quantification of *Clavibacter michiganensis* subsp. *michiganensis* in tomato plants and seeds.

consist of small, dark lesions surrounded by white halos. Foliar symptoms occasionally include small, white, blister-like spots. More commonly, yellow-to-brown regions of marginal necrosis, sometimes referred to as “firing,” develop on the leaflets of diseased plants (14). Another symptom of this disease is internal necrosis called “stem discoloration” (Fig. 3A and B). During the last stage of infection, the whole plant wilts and death is observed (Fig. 3D and E).

Disease management. Disease management strategies can be grouped into four categories: exclusion, eradication, avoidance, and control. Chemical, biological, physical, and genetic methods of disease management are all part of the control strategy. *Clavibacter* can be excluded from the farming environment by hygienic measures such as the use of clean propagation materials, plant materials, water, and humans. The Good Seed and Plant Practices (GSPP, <http://www.gspp.eu/>) organization founded in the Netherlands and France aims to prevent pathogen infection of tomato seed and plant lots. The use of GSPP and the application of control agents is expected to strongly support the commercial production of pathogen-free seed and planting material.

Antimicrobial compounds such as copper sulfate, copper hydroxide, copper hydroxide/mancozeb, streptomycin, and streptomycin/copper hydroxide are known to reduce disease incidence; however, currently, no chemicals can satisfactorily control the pathogen (26), and the use of copper compounds can result in phytotoxic effects (104). Some organic antimicrobial substances, such as lysozyme, fragarin (36), endolysins of bacteriophages (101), and plant essential oils (24,60) can also reduce bacterial spread.

Another method of controlling *clavibacter* in host plants is chemical activation of the plant defense system. The activation of

constitutive defenses can result in preformed physical or chemical barriers against pathogen invasion. Additionally, the host can be primed to generate antimicrobial compounds in response to specific elicitors that are produced by an invading pathogen. Chemicals that have been described as activating plant resistance are salicylic acid (SA), jasmonic acid (JA), DL- β -aminobutyric acid (BABA) (7), potassium salts, 2,6-dichloroisonicotinic acid (INA), acibenzolar-*S*-methyl, and specific volatiles such as nitric oxide and ethylene (8).

Attempts have been made to control *clavibacter* using biocontrol agents. Treatment with *B. subtilis* (93) or preinoculation with avirulent pathogen (69) reduces bacterial spread, but the mechanisms by which *clavibacter* is inhibited on tomatoes is unknown. Growing practices such as lowering the pH of the growth solution (nutrients) can also limit the growth of bacteria (49). Although significant levels of control were reported for chemical and biological treatments, such treatments were not economically feasible and are not widely used.

Resistant cultivars would be an effective method of control and would result in a reduction of the use of chemicals; however, no commercial cultivars with substantial levels of resistance to the pathogen are on the market yet. As a result, there should be a zero tolerance of *clavibacter* in the commercial seed trade.

Sources of disease resistance. Several *Solanum* species have been found to be resistant to *clavibacter*. Resistance was first reported in *S. pimpinellifolium* (L.) Mill. (Anonymous, 1934 cited in [90]), and later it was reported in several new *S. pimpinellifolium* (82,85,86,90,92) and other species such as *S. habrochaites* (39,46, 85,86), *S. lycopersicum* derived lines (23,32,33,38,42,61,62,73,91), *S. arcanum* (65,86), and *S. chilense* (85,86). Resistant *S. lyco-*

Fig. 3. Common symptoms of bacterial canker disease: **A and B**, stem discoloration, **C**, unilateral wilting, **D and E**, dead tomato plants due to bacterial canker, and **F and G**, stem canker.

persicum varieties such as Bulgaria 12 (32), Heinz 2990 (33), and Okitsu sozai 1-20 (61) based on resistance from *S. pimpinellifolium* were not commercially successful, and the resistance could not be transferred to other varieties due to the complexity of the genetic background of the resistance.

Genetic analysis of resistance. Studies have shown that pathogen resistance is mostly polygenic (90,93) (Table 1). Backcross and F₂ populations between resistant and susceptible *S. arcanum* accessions revealed two or three recessive genes that are involved in resistance (64). An analysis of three resistant *S. lycopersicum* breeding lines (derived from crosses between the susceptible *S. lycopersicum* and the resistant *S. chilense* or *S. peruvianum* accessions) identified a single dominant gene on chromosome 4 and a few modifier genes (99). Progeny plants obtained after the crossing of two moderately resistant *S. lycopersicum* parents showed higher levels of resistance than either parent (73). Transgressive segregation was also observed following crossing of *S. lycopersicum* and *S. peruvianum* resistance sources (23). All the mentioned studies were performed without molecular marker technology. The resistance of *S. arcanum* LA2157 was studied in an intraspecific cross, and five quantitative trait loci (QTL) were identified on chromosomes 1, 6, 7, 8, and 10 (77). This same resistance source was used for an interspecific cross with *S. lycopersicum*, and 3 QTLs (on chromosomes 5, 7, and 9) were identified in an F₂ population. These resistance loci were additive and co-dominant, and combining the main QTL on chromosome 7 with another QTL gave a resistance level similar to that of the resistant *S. peruvianum* LA2157 parent (94). Another well-characterized source of resistance is *S. habrochaites* LA407. Genetic analysis of an inbred backcross population (IBC) resulted in the identification of two QTLs on chromosomes 2 and 5 (54). Fine mapping of these QTLs identified a 4.4 cM interval on chromosome 2 (Rcm 2.0) and a 2.2 cM interval on chromosome 5 (Rcm 5.1), and the QTLs interacted additively (21). Resistance genes also show different types of interaction, e.g., additive, incomplete dominance, and modifying effects. The dominant or recessive resistance type can also change depending on the genetic background, as for the resistance of *S. arcanum* LA2157 (77,94). Differences in the interactions between resistance genes under different environmental conditions are a further complicating factor.

Morphological resistance. Stem morphologies and the vascular systems vary among commercial tomato cultivars and their related wild species. A study of the vascular structure of both wild and domesticated tomatoes, as well as populations derived from these

species, revealed several characteristics that may have a role in resistance to *Clavibacter* (21). The hypocotyl region vascular structure can vary from square to circular. The time period required for the root to stem vascular transition in early development is different between *S. habrochaites* and *S. lycopersicum*; in *S. habrochaites* and cherry-type tomatoes, this time period is shorter. Additionally, the primary vascular bundles and secondary vascular tissues are thicker in *S. habrochaites*. A genetic analysis of an F₂ population showed a significant association of a region on chromosome 2 with the size of the primary vascular bundles, the shape of the vascular system, and the thickness of the secondary vascular tissue. *S. habrochaites* plants homozygous for this region had longer primary vascular bundles, thicker secondary vascular tissue, and a triangular stem shape. This same region on chromosome 2 has also been associated with pathogen resistance and other morphological characteristics. The shape and thickness of vascular bundles and faster vascular growth results in stronger vascular tissue and may play a role in resisting cell wall-degrading enzymes that are produced by the pathogen. Based on this study, we suggest that the morphological differences between resistant wild species of tomatoes and commercial cultivars are involved in bacterial resistance. In our studies, we observed wilting symptoms in young *S. pimpinellifolium* plants that disappeared in adult plants; however, this was not observed in *S. lycopersicum*, for which wilting continued as the plant developed (82).

Temperature, plant age, resistance, and inoculum concentration have roles in the disease incubation period and development. Generally, as plants age, the incubation period of pathogens increases. The incubation period also increases with cooler temperatures, and disease development is also slowed. Until a certain threshold is met, the bacterial inoculum concentration is negatively correlated with incubation period and positively correlated with disease severity. Obviously, high host plant resistance levels lead to an increased incubation period and a decrease in disease severity (19).

Host-Pathogen Interactions

The pathogen. The ability of the pathogen to invade a host can be separated into colonization and virulence. The pathogen behaves as an endophyte and attempts to colonize the host by spreading within, and retrieving nutrients from, the xylem tissue. Fifty-seven ABC transporter pathogen proteins have been identified in infected plants (79); these proteins, belonging to the Ppa family of serine proteases and the subtilase family, are found in xylem sap and are thought to play a role in plant colonization (79). A mutant

Table 1. Sources of resistance to *Clavibacter michiganensis* subsp. *michiganensis* and their genetic interactions in *Solanum* species

Resistance source	Population type	Gene(s) interactions	Reference
<i>S. lycopersicum</i> <i>S. pimpinellifolium</i> <i>S. habrochaites</i>	<i>S. lycopersicum</i> lines Bulgaria 12, Homestead, Heinz 1350, Highlander and Campbell <i>S. pimpinellifolium</i> Utah 20 <i>S. habrochaites</i> PI251305	Polygenic and horizontal type resistance (regardless of strains)	(88,91)
<i>S. lycopersicum</i> line Bulgaria 12	F2 and backcross	Incomplete dominant genes with one to four major genes	(88)
<i>S. lycopersicum</i> lines Hawaii 7998 and Irat-L3	RIL population	Complementary genes with transgressive segregation	(75)
<i>S. pimpinellifolium</i> Utah 737 and Utah 20	F2 and backcross of interspecific cross	The estimated gene number ranges from 4 to 11 with presence of modifying genes	(25)
<i>S. peruvianum</i> var. <i>humifusum</i>	F2 and backcross population of resistant three-genome hybrid <i>S. lycopersicum</i> Line Cm 180 [<i>S. peruvianum</i> var. <i>humifusum</i> × (<i>S. lycopersicum</i> × <i>S. chilense</i> LA 460)]	A single dominant gene on Chr 4.	(98)
<i>S. arcanum</i> LA2157	F2 and backcross of intraspecific cross	Two to three genes with recessive inheritance	(66)
<i>S. arcanum</i> LA2157	Backcross of intraspecific cross	5 regions on chromosomes 1, 6, 7, 8, and 10	(79)
<i>S. arcanum</i> LA2157	F2 population of interspecific cross	3 QTLs on chromosomes 5, 7, and 9. Additive interactions of QTLs	(93)
<i>S. habrochaites</i> LA407	Inbred backcross lines of interspecific cross	2 QTLs on chromosome 2 and 5. Additive interactions of QTLs	(55)

clavibacter strain lacking a 129-kb chromosomal region had impaired virulence and was unable to effectively colonize its host (43). Genome sequencing of *Clavibacter lookalike* strains showed that they were missing most of the genes located in the pathogenicity island (PAI) of clavibacter (105).

The PAI of clavibacter has a low G+C content and is divided into two subregions: Chp and TomA. ChpC was the first gene to be identified in this island as having a role in host colonization (44,87). The tomatinase gene tomA, also located in the PAI region, is responsible for the breakdown of the secondary plant metabolite α -tomatine. This metabolite is a basal defense component of tomatoes (56) and reduces the population of fungi (76) and bacteria (2). The concentration of α -tomatine rises after pathogen attack (2); however, the role of tomA is unclear, as a tomA mutant strain was as virulent as the nonmutant strain (56). Although the role of α -tomatine in host defense is unclear, the quantity of α -tomatine may play a role in the basal or activated defense systems. When the Chp/TomA region is absent, host defense genes are induced at an early stage of infection, suggesting that this region is involved in the suppression of host defenses. The tomA subregion does not appear to be involved in pathogenicity (56), but this subregion may allow the pathogen to obtain plant nutrients (37). Microorganisms, mainly bacteria, produce extracellular polymeric substance (EPS), which is a complex mixture of biopolymers consisting of polysaccharides, proteins, nucleic acids, lipids, and humic substances. EPS enables bacteria to attach to certain substrates and is protective against environmental stress and dehydration (98). The clavibacter EPS consists of L-fructose, D-galactose, D-glucose, pyruvate, succinate, and acetate in a 2:1:1:1:0.5:1.5 ratio (96). Endophyte strains producing identical quantities of EPS with the same composition did not cause wilting, and a mutant clavibacter strain producing a reduced amount of EPS was differentially virulent (11). Even strains producing only 10% of the level of EPS of a normal strain were as virulent as nonmutant strains. Nonpathogenic *Clavibacter lookalike* strains can have functional EPS coding genes (105). Obviously, a clear role for EPS has not been experimentally proven; it is not a virulence factor that is directly involved in pathogenicity, but it is thought to be involved in colonization (37) through the protection of bacterial cells by generating a protective biofilm (17). Genes encoding surface proteins may be responsible for the production of such a biofilm (37).

Although bacteria are single cells, their gene expression is influenced by cell population density via a phenomenon known as quorum sensing, which enables the bacteria to adapt to changing conditions (48). We believe that *C. michiganensis* subsp. *michiganensis* changes its behavior from endophytic to pathogenic via quorum-sensing dependent phenomenon when the pathogen population reaches a 10^8 CFU/g plant material threshold in tomatoes (29). Several bacterial proteins have been identified with a putative role in signal transduction (79); however, all of these proteins were also found in *Clavibacter lookalike* strains (105). Genes encoding extracellular enzymes are necessary for degrading xylem cell walls; these genes are upregulated during the early stages of infection (17) and later down regulated (37). Therefore, these genes are thought to trigger an early signal cascade that is required for symptom development at later stages of infection. The pathogen has a 3.2-Mb genome and two plasmids, pCM1 (27 kb) and pCM2 (70 kb) (43). Using deletion mutation and complementation analysis, two virulence factors, CeIA on pCM1 (53), and PatI on pCM2 (29), have been identified. The CeIA gene has a 2.4-kb coding region encoding an endo- β -1,4-glucanase. This 78-kDa protein consists of three domains: a catalytic domain, a type II-like domain, and a C-terminal domain. The CeIB gene, a homolog of CeIA lacking the third domain, is present on the chromosome. The PatI gene putatively encodes a 280 amino acid (29.7 kDa) serine protease, and has two homologs on pCM2, phpA and phpB (13). Proteins from both of these genes contain an N-terminal signal peptide and are secreted (30), and both genes are required for the development of fully diseased plants. Genome sequencing and annotation of *C. michiganensis* subsp. *michiganensis* revealed six

additional PatI homologs in the PAI region, termed ChpB, C, D, E, F, and G. These homologous genes code for serine proteases (58); however, ChpA, B, and D are nonfunctional genes due to frame shifts (44) and are considered to be pseudogenes (79). *Clavibacter lookalike* strains isolated from tomatoes were missing both these virulence factors and homologous genes (105). Another putative virulence gene encoding a perforin protein may permit bacteria to manipulate host genes by delivering bacterial effectors into host cells. This mechanism is comparable to the upregulation of type-III secretion systems following infection by gram-negative bacteria (37). Many additional genes encoding cell-wall degrading enzymes were identified in the PAI region (79). Pathogenic bacteria did induce ethylene response genes (79), and ethylene is considered to be required for virulence. Other virulence factors, Vatr1 and Vatr2, have been identified as being involved in wilting and ethylene synthesis (78). Virulence factor expression levels can change depending upon the age of the plant. If plants are infected when they are most vulnerable, the expression levels of virulence genes are higher compared with plants that were infected after this period (83). Wilting begins when clavibacter transitions from endophytic to pathogenic. The cause of wilting is thought to be vessel plugging, toxins, or both; however, no vessel plugging material was found in pathogen-invaded wilting plants (100), thus the more the likely cause of wilting is the presence of toxins.

Transcriptional analysis of wild-type *C. michiganensis* subsp. *michiganensis* and *C. michiganensis* subsp. *michiganensis* lacking both plasmids revealed the interplay of chromosomal and plasmid genes (16,79). The plasmid-based expression of ceIA and PatI was reduced in the absence of the PAI chromosomal region, and the expression of ChpC and PpaA, representing two different serine protease families, was reduced in the absence of the plasmids (16). This interplay of expression is thought to be necessary for successful colonization, as strains of *C. michiganensis* subsp. *michiganensis* lacking the Chp/TomA region or one of the plasmids were unable to colonize a host effectively, and bacteria were only found in the area of inoculation (17).

The plant. For the tomato-clavibacter interaction, the putative PAMP proteins were identified based on their upregulation following infection. Multiple defense related proteins covering different functions and metabolic pathways are induced after infection. Several protein phosphatases that activate signal transduction cascades, kinases that are involved in defense mechanisms against bacteria, and activation of transcription factors of several classes were detected in the plant following pathogen infection (79). The basal host plant defense was activated following infection and includes defense-related genes, the production and scavenging of free oxygen radicals, enhanced protein turnover, and hormone synthesis (4,79). Many pathogen-related (PR) proteins that possess antimicrobial activity have been identified after infection (79). Additionally, down regulation of some defense related genes such as class III peroxidases was observed during the pathogen-host interaction, indicating the suppression of the plant defense system by the pathogen (4,79). The down regulation of some metabolic pathways such as photosynthesis and carbon metabolism, and upregulation of senescence-associated proteins, occurs following infection (22). Gene expression studies at 4 days after clavibacter inoculation but before visible symptoms, and at 8 days when wilting occurred, revealed that 122 of the 9,254 examined plant genes were differentially expressed at at least one time point (4). The majority of the genes induced by clavibacter during the early stage of infection were also differentially expressed during the later stages of disease development.

A significant change in the expression of genes involved in ethylene synthesis was observed after pathogen infection; however, in the early stage of pathogenesis, no significant ethylene emission was detected, but was detected in later stages. There was a delay in the wilting of ethylene-insensitive never ripe (Nr) mutant tomato plants and in ethylene-deficient mutants, so it appears that ethylene does play a role in disease progress, although the mechanism is unknown (4). The most abundantly expressed proteins following

infection were enzymes involved in methionine metabolism and ethylene biosynthesis (79). As ethylene is thought to be involved in plant susceptibility to infection (95), it is speculated that the early induction of ethylene synthesis in tomatoes by *Clavibacter* results in the senescence of vascular tissue and easier penetration of the pathogen. One hundred and sixty genes that are known to be involved in defense-related signaling and were either down- or up-regulated during the tomato-*Pseudomonas syringae* pv. *tomato* interaction were tested for response in *Nicotiana benthamiana* plants inoculated with *Clavibacter*. The genes were individually silenced, and the silencing of six genes including StSN2 and ELP resulted in a significantly higher bacterial titer and faster wilting (5). The overexpression of these two genes in tomatoes enhanced their tolerance, as observed by a significant delay of symptoms, a reduction in lesion size at the point of inoculation, and a lower bacterial population (6). The StSN2 gene encodes the snaking-2 (SN2) cysteine-rich peptide that is considered to be antimicrobial, and the extension-like protein gene (ELP) encodes a cell-wall hydroxyproline-rich glycoprotein. These two genes were also differentially regulated during tomato plant infection with *Clavibacter* (4).

Two tomato lines, with the QTL regions Rcm 2.0 and Rcm 5.1, that showed significantly less wilting and bacterial growth were used for a proteomic analysis at 72 and 144 h postinoculation. In total, 42 differentially expressed proteins were identified, of which 26 could be annotated. Those proteins were involved in the defense and stress response, protein regulation, protein synthesis and processing, energy production, and metabolism. Lines containing Rcm 2.0 and 5.1 produced distinct proteins and had a reduced pathogen population. The accumulation of these specific proteins was dependent upon the genotype and the length of time following inoculation. It is thought that the two QTLs act through different mechanisms in response to *Clavibacter* (22).

Conclusion

C. michiganensis subsp. *michiganensis* is still the most dangerous bacterium infecting tomatoes, and despite management and breeding efforts, disease outbreaks occur continuously. Bioassays are still the most reliable method for detecting the bacteria; however, the development of fast, sensitive, and reliable detection methods is still needed. The Ptssk primer-based method seems to be the most reliable, and with our adaption it can be used to detect and quantify bacteria at a sensitivity level of 10^2 CFU/ml. Detection and quantification is needed for evaluating plant material in breeding programs. Reliable and more sensitive serological methods such as ELISA are preferred in the field for recognizing bacterial canker as soon as possible.

Symptoms of infection become visible when the bacteria have already spread throughout the plant, and at this stage it is too late to stop the disease using chemical treatments or to remove the plants. The best management method is to prevent the presence of bacteria through clean agriculture as indicated by the Good Seed and Plant Practices (GSPP) organization. An effective method for controlling *Clavibacter* is the use of resistant varieties; however, to date, wild tomato species are the only resistance sources. It is very important for breeders to know how resistance mechanisms work in order to develop resistant varieties. Using wild species as a resistance source may have unwanted side-effects due to either the resistance mechanism itself or linkage drag.

Clavibacter seems to have acquired pathogenicity via horizontal gene transfer, and the low G+C content regions that contain virulence factors play an important role in colonization and virulence. *C. michiganensis* subsp. *michiganensis* strains lacking plasmids are able to colonize the host, but are unable to establish pathogenicity. *Clavibacter lookalike* strains lacking the low G+C content regions and lacking the plasmids are not able to successfully colonize tomatoes. Consequently, the low G+C content regions are thought to play a role in colonization, and the virulence factors on the plasmids play a role in pathogenicity. Interactions between the regions with low G+C content and the plasmid virulence factors may also

affect the level of pathogenicity. The roles of EPS and many putative virulence factors in virulence remain to be experimentally determined. The exact plant responses to the pathogen during colonization and becoming pathogenic have yet to be determined. It is obvious that different plant mechanisms are involved in host defense. It is not known whether the resistance factors are antimicrobial substances and/or morphological barriers such as rigid vascular tissues. In this review, we hypothesized with regards to the plant resistance mechanism, but experimental research is needed to prove it. Understanding the pathogen interaction with tomatoes will provide new perspectives for improved management of this disease. As the pathogen strains are diverse, it is important to determine their mechanisms of pathogenicity and whether they differ from strain to strain. Understanding the host responses to *Clavibacter*, which may vary among tomato species, will give us insight as to why plants are resistant. Use of both of the general concepts of host-pathogen interactions for specific studies into the interaction of *Clavibacter* and tomatoes will be essential to identify gene(s) involved in resistance, and it is hoped that that information will lead to the generation of tomato varieties that are resistant to *Clavibacter*.

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Literature Cited

- Alvarez, A. M., Kaneshiro, W. S., and Vine, B. G. 2004. Diversity of *Clavibacter michiganensis* subsp. *michiganensis* populations in tomato seed: What is the significance. *Acta Hortic.* (ISHS) 695:205-214.
- Arwiyanto, T., Sakata, K., Goto, M., Tsuyumu, S., and Takikawa, Y. 1994. Induction of tomatine in tomato (*Lycopersicon esculentum*) by an avirulent strain of *Pseudomonas solanacearum*. *Ann. Phytopathol. Soc. Jpn.* 60:288-294.
- Bach, H. J., Jessen, I., Schlöter, M., and Munch, J. C. 2003. A TaqMan-PCR protocol for quantification and differentiation of the phytopathogenic *Clavibacter michiganensis* subspecies. *J. Microbiol. Methods* 52:85-91.
- Balaji, V., Mayrose, M., Sherf, O., Jacob-Hirsch, J., Eichenlaub, R., Iraki, N., Manulis-Sasson, S., Rechavi, G., Barash, I., and Sessa, G. 2008. Tomato transcriptional changes in response to *C. michiganensis* subsp. *michiganensis* reveal a role for ethylene in disease development. *Plant Physiol.* 146:1797-1809.
- Balaji, V., Sessa, G., and Smart, C. D. 2011. Silencing of host basal defense response-related gene expression increases susceptibility of *Nicotiana benthamiana* to *C. michiganensis* subsp. *michiganensis*. *Phytopathology* 101:349-357.
- Balaji, V., and Smart, C. D. 2012. Over-expression of snakin-2 and extensin-like protein genes restricts pathogen invasiveness and enhances tolerance to *C. michiganensis* subsp. *michiganensis* in transgenic tomato (*Solanum lycopersicum*). *Transgenic Res.* 21:23-37.
- Baysal, O., Gursoy, Y., Ormek, H., and Duru, A. 2005. Induction of oxidants in tomato leaves treated with DL-beta-amino butyric acid (BABA) and infected with *Clavibacter michiganensis* ssp. *michiganensis*. *Eur. J. Plant. Pathol.* 112:361-369.
- Baysal, O., Soyulu, E. M., and Soyulu, S. 2003. Induction of defence-related enzymes and resistance by the plant activator acibenzolar-S-methyl in tomato seedlings against bacterial canker caused by *Clavibacter michiganensis* ssp. *michiganensis*. *Plant Pathol.* 52:747-753.
- Bella, P., Ialacci, G., Licciardello, G., La Rosa, R., and Catara, V. 2012. Characterization of atypical *C. michiganensis* subsp. *michiganensis* populations in greenhouse tomatoes in Italy. *J. Plant Pathol.* 94:635-642.
- Berendsen, S. M. H., Koenraadt, H., Woudt, B., and Oosterhof, J. 2011. The development of a specific Real-Time TaqMan for the detection of *Clavibacter michiganensis* subsp. *michiganensis*. *APS-IPPC Meeting*, Honolulu, Hawaii.
- Bermopohl, A., Drier, J., Bahro, R., and Eichenlaub, R. 1996. Exopolysaccharides in the pathogenic interaction of *C. michiganensis* subsp. *michiganensis* with tomato plants. *Microbiol. Res.* 151:391-399.
- Bolkan, H. A., Waters, C. M., and Fatmi, M. 1996. ISTA handbook on seed health testing. Zurich, Switzerland. ISTA sheet no. 67.
- Burger, A., Grafen, I., Engemann, J., Niermann, E., Pieper, M., Kirchner, O., Gartemann, K. H., and Eichenlaub, R. 2005. Identification of homologues to the pathogenicity factor Pat-1, a putative serine protease of *C. michiganensis* subsp. *michiganensis*. *Microbiol. Res.* 160:417-427.
- Carlton, W. M., Braun, E. J., and Gleason, M. L. 1998. Ingress of *Clavibacter michiganensis* subsp. *michiganensis* into tomato leaves through hy-

- dathodes. *Phytopathology* 88:525-529.
15. Cass Smith, W. P., and Goss, M. 1946. Bacterial canker of tomatoes. *J. Dep. Agric. Western Aust.* 23:147-156.
 16. Chalupowicz, L., Cohen-Kandli, M., Dror, O., Eichenlaub, R., Gartemann, K. H., Sessa, G., Barash, I., and Manulis-Sasson, S. 2010. Sequential expression of bacterial virulence and plant defense genes during infection of tomato with *C. michiganensis* subsp. *michiganensis*. *Phytopathology* 100:252-261.
 17. Chalupowicz, L., Zellermann, E. M., Fluegel, M., Dror, O., Eichenlaub, R., Gartemann, K. H., Savidor, A., Sessa, G., Raki, N., Barash, I., and Manulis-Sasson, S. 2012. Colonization and movement of GFP-Labeled *Clavibacter michiganensis* subsp. *michiganensis* during tomato infection. *Phytopathology* 102:23-31.
 18. Chang, R. J., Ries, S. M., and Pataky, J. K. 1991. Dissemination of *Clavibacter michiganensis* subsp. *michiganensis* by practices used to produce tomato transplants. *Phytopathology* 81:1276-1281.
 19. Chang, R. J., Ries, S. M., and Pataky, J. K. 1992. Effects of temperature, plant-age, inoculum concentration, and cultivar on the incubation period and severity of bacterial canker of tomato. *Plant Dis.* 76:1150-1155.
 20. Chun, W. C. C. 1982. Identification and detection of *Corynebacterium michiganense* in tomato seed using the indirect enzyme-linked immunosorbent assay. M.Sc. thesis. University of Hawaii, Honolulu, HI.
 21. Coaker, G., and Francis, D. 2004. Mapping, genetic effects, and epistatic interaction of two bacterial canker resistance QTLs from *Lycopersicon hirsutum*. *Theor. Appl. Genet.* 108:1047-1055.
 22. Coaker, G. L., Willard, B., Kinter, M., Stockinger, E. J., and Francis, D. M. 2004. Proteomic analysis of resistance mediated by Rcm 2.0 and Rcm 5.1, two loci controlling resistance to bacterial canker of tomato. *Mol. Plant-Microbe Interact.* 17:1019-1028.
 23. Crinò, P., Veronesi, P., Stamigna, C., Chiaretti, D., Lai, A., Bitti, M. E., and Saccardo, F. 1995. Breeding for resistance to bacterial canker in Italian tomatoes for fresh market. *Acta Hort.* (ISHS) 412:539-545.
 24. Daferera, D. J., Ziogas, B. N., and Polissiou, M. G. 2002. The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp. and *C. michiganensis* subsp. *michiganensis*. *Crop Prot.* 22:39-44.
 25. de Leon, L., Rodriguez, A., Lopez, M. M., and Siverio, F. 2008. Evaluation of the efficacy of immunomagnetic separation for the detection of *C. michiganensis* subsp. *michiganensis* in tomato seeds. *J. Appl. Microbiol.* 104:776-786.
 26. de Leon, L., Siverio, F., Lopez, M. M., and Rodriguez, A. 2008. Comparative efficiency of chemical compounds for in vitro and in vivo activity against *Clavibacter michiganensis* subsp. *michiganensis*, the causal agent of tomato bacterial canker. *Crop Prot.* 27:1277-1283.
 27. de Leon, L., Siverio, F., and Rodriguez, A. 2006. Detection of *Clavibacter michiganensis* subsp. *michiganensis* in tomato seeds using immunomagnetic separation. *J. Microbiol. Methods* 67:141-149.
 28. Dreier, J., Bermpohl, A., and Eichenlaub, R. 1995. Southern hybridization and PCR for specific detection of phytopathogenic *C. michiganensis* subsp. *michiganensis*. *Phytopathology* 85:462-468.
 29. Dreier, J., Meletzus, D., and Eichenlaub, R. 1997. Characterization of the plasmid encoded virulence region pat-1 of phytopathogenic *C. michiganensis* subsp. *michiganensis*. *Mol. Plant-Microbe Interact.* 10:195-206.
 30. Eichenlaub, R., and Gartemann, K. H. 2011. The *Clavibacter michiganensis* subspecies: Molecular investigation of gram-positive bacterial plant pathogens. *Annu. Rev. Phytopathol.* 49:445-464.
 31. Eichenlaub, R., Gartemann, K. H., and Burger, A. 2006. *Clavibacter michiganensis*, a group of gram-positive phytopathogenic bacteria. Pages 385-421 in: *Plant-Associated Bacteria*. Springer, the Netherlands.
 32. Elenkov, E. 1965. Die Selektion von tomaten auf resistenz gegen die bakterienwelke. *Int. Z. Landwirt.* 594-597.
 33. Emmatt, D. A., and John, C. A. 1973. Evaluation of resistance to bacterial canker of H2990, a new tomato variety. *Plant Dis. Rep.* 57:584-586.
 34. Fatmi, M., and Schaad, N. W. 1988. Semiselective agar medium for isolation of *Clavibacter michiganense* subsp. *michiganense* from tomato seed. *Phytopathology* 78:121-126.
 35. Fatmi, M., and Schaad, N. W. 2002. Survival of *Clavibacter michiganensis* ssp. *michiganensis* in infected tomato stems under natural field conditions in California, Ohio and Morocco. *Plant Pathol.* 51:149-154.
 36. Filippone, M. P., Diaz-Ricci, J. C., Castagnaro, A. P., and Farias, R. N. 2001. Effect of fragarin on the cytoplasmic membrane of the phytopathogenic *Clavibacter michiganensis*. *Mol. Plant-Microbe Interact.* 14:925-928.
 37. Flugel, M., Becker, A., Gartemann, K. H., and Eichenlaub, R. 2012. Analysis of the interaction of *Clavibacter michiganensis* subsp. *michiganensis* with its host plant tomato by genome-wide expression profiling. *J. Biotechnol.* 160:42-54.
 38. Forster, R. L., and Echandi, E. 1973. Relation of age of plants, temperature, and inoculum concentration to bacterial canker development in resistant and susceptible *Lycopersicon* spp. *Phytopathology* 63:773-777.
 39. Francis, D. M., Kabelka, E., Bell, J., Franchino, B., and Clair, D. S. 2001. Resistance to bacterial canker in tomato (*Lycopersicon hirsutum* LA407) and its progeny derived from crosses to *L. esculentum*. *Plant Dis.* 85:1171-1176.
 40. Franken, A. A. J. M., Kamminga, G. C., Snijders, W., Van Der Zouwen, P. S., and Birnbaum, Y. E. 1993. Detection of *Clavibacter michiganensis* ssp. *michiganensis* in tomato seeds by immunofluorescence microscopy and dilution plating. *Eur. J. Plant Pathol.* 99:125-137.
 41. Ftayeh, R. M., von Tiedemann, A., and Rudolph, K. W. E. 2011. A new selective medium for isolation of *C. michiganensis* subsp. *michiganensis* from tomato plants and seed. *Phytopathology* 101:1355-1364.
 42. Gardner, R. G., Shoemaker, P. B., and Echandi, E. 1990. Evaluation of tomato lines for resistance to bacterial canker in North Carolina. *Tomato Genet. Coop. Rep.* 40:10-12.
 43. Gartemann, K.-H., Abt, B., Bekel, T., Burger, A., Engemann, J., Flügel, M., Gaigalat, L., Goesmann, A., Gräfen, I., Kalinowski, J., Kaup, O., Kirchner, O., Krause, L., Linke, B., McHardy, A., Meyer, F., Pohle, S., Rückert, C., Schneiker, S., Zellermann, E.-M., Pühler, A., Eichenlaub, R., Kaiser, O., and Bartels, D. 2008. The genome sequence of the tomato-pathogenic Actinomycete *C. michiganensis* subsp. *michiganensis* NCPPB382 reveals a large island involved in pathogenicity. *J. Bacteriol.* 190:2138-2149.
 44. Gartemann, K.-H., Kirchner, O., Engemann, J., Gräfen, I., Eichenlaub, R., and Burger, A. 2003. *C. michiganensis* subsp. *michiganensis*: First steps in the understanding of virulence of a Gram-positive phytopathogenic bacterium. *J. Biotechnol.* 106:179-191.
 45. Hadas, R., Kritzman, G., Klietman, F., Gefen, T., and Manulis, S. 2005. Comparison of extraction procedures and determination of the detection threshold for *Clavibacter michiganensis* ssp. *michiganensis* in tomato seeds. *Plant Pathol.* 54:643-649.
 46. Hassan, A. A., Strider, D. L., and Konsler, T. L. 1968. Application of cotyledonary symptoms in screening for resistance of tomato to bacterial canker and host range studied. *Phytopathology* 58:233-239.
 47. Hausbeck, M. K., Bell, J., Medina-Mora, C., Podolsky, R., and Fulbright, D. W. 2000. Effect of bactericides on population sizes and spread of *C. michiganensis* subsp. *michiganensis* on tomatoes in the greenhouse and on disease development and crop yield in the field. *Phytopathology* 90:38-44.
 48. Henke, J. M., and Bassler, B. L. 2004. Bacterial social engagements. *Trends Cell Biol.* 14:648-656.
 49. Huang, R., and Tu, J. C. 2001. Effects of nutrient solution pH on the survival and transmission of *Clavibacter michiganensis* ssp. *michiganensis* in hydroponically grown tomatoes. *Plant Pathol.* 50:503-508.
 50. International Seed Federation (ISF). 2011. Method for detection of *C. michiganensis* subsp. *michiganensis* on tomato seed. International Seed Federation Report.
 51. International Seed Federation (ISF). 2008. Protocol for the detection of *C. michiganensis* subsp. *michiganensis* on tomato seeds by isolation on media version 3.
 52. Jacques, M.-A., Durand, K., Orgeur, G., Balidas, S., Fricot, C., Bonneau, S., Quillévère, A., Audusseau, C., Olivier, V., Grimault, V., and Mathis, R. 2012. Phylogenetic analysis and polyphasic characterization of *Clavibacter michiganensis* strains isolated from tomato seeds reveal that nonpathogenic strains are distinct from *C. michiganensis* subsp. *michiganensis*. *Appl. Environ. Microbiol.* 78:8388-8402.
 53. Jahr, H., Dreier, J., Meletzus, D., Bahro, R., and Eichenlaub, R. 2000. The endo-beta-1,4-glucanase CelA of *Clavibacter michiganensis* subsp. *michiganensis* is a pathogenicity determinant required for induction of bacterial wilt of tomato. *Mol. Plant-Microbe Interact.* 13:703-714.
 54. Kabelka, E., Franchino, B., and Francis, D. M. 2002. Two loci from *Lycopersicon hirsutum* LA407 confer resistance to strains of *Clavibacter michiganensis* subsp. *michiganensis*. *Phytopathology* 92:504-510.
 55. Kaneshiro, W. S., Mizumoto, C. Y., and Alvarez, A. M. 2006. Differentiation of *Clavibacter michiganensis* subsp. *michiganensis* from seed-borne saprophytes using ELISA, Biolog and 16S rDNA sequencing. *Eur. J. Plant Pathol.* 116:45-56.
 56. Kaup, O., Grafen, I., Zellermann, E. M., Eichenlaub, R., and Gartemann, K. H. 2005. Identification of a tomatinase in the tomato-pathogenic actinomycete *C. michiganensis* subsp. *michiganensis* NCPPB382. *Mol. Plant-Microbe Interact.* 18:1090-1098.
 57. Kawaguchi, A., Tanina, K., and Inoue, K. 2010. Molecular typing and spread of *Clavibacter michiganensis* subsp. *michiganensis* in greenhouses in Japan. *Plant Pathol.* 59:76-83.
 58. Kleitman, F., Barash, I., Burger, A., Iraki, N., Falah, Y., Sessa, G., Weinthal, D., Chalupowicz, L., Gartemann, K. H., Eichenlaub, R., and Manulis-Sasson, S. 2008. Characterization of a *Clavibacter michiganensis* subsp. *michiganensis* population in Israel. *Eur. J. Plant Pathol.* 121:463-475.
 59. Kontaxis, D. G. 1962. Leaf trichomes as avenues for infection by *Corynebacterium michiganense*. *Phytopathology* 52:1306-1307.
 60. Kotan, R., Cakir, A., Ozer, H., Kordali, S., Cakmakci, R., Dadasoglu, F., Dikbas, N., Aydin, T., and Kazaz, C. 2014. Antibacterial effects of *Origanum onites* against phytopathogenic bacteria: Possible use of the extracts from protection of disease caused by some phytopathogenic bacteria. *Sci. Hortic.* 172:210-220.
 61. Kuriyama, T., and Kuniyasu, K. 1974. Studies on the breeding of resistant tomato by interspecific hybridization. III. On the breeding of a new tomato line resistant to bacterial canker caused by *Corynebacterium michiganense*. *Bull. Vege. Ornamental Crops Res. Stn. Jpn. A* 1:93-107.
 62. Laterot, H., Brand, R., and Daunay, M. C. 1978. La resistance à *Corynebacterium michiganense* chez la tomate. Étude bibliographique. *Ann. Amélior. Plantes* 28:579-591.

63. Lelis, F. V., Czajkowski, R., Souza, R., Ribeiro, D., and Wolf, J. 2014. Studies on the colonization of axenically grown tomato plants by a GFP-tagged strain of *C. michiganensis* subsp. *michiganensis*. Eur. J. Plant Pathol. 139:53-66.
64. Lindhout, P., and Purimahu, C. 1989. Investigations on tomato breeding for resistance to *Corynebacterium michiganense* Pages 16-18 in: Book of Poster Abstracts, Part II. XIIth EUCARPIA Congress, Science for Plant Breeding, Göttingen, Germany.
65. Lindhout, P., and Purimahu, C. 1987. Resistance against *Corynebacterium michiganense* found in *Lycopersicon peruvianum*. 10th Meeting Eucarpia Tomato Working Group, Pontecagnano, Italy.
66. Luo, L. X., Walters, C., Bolkan, H., Liu, X. L., and Li, J. Q. 2008. Quantification of viable cells of *Clavibacter michiganensis* subsp. *michiganensis* using a DNA binding dye and a real-time PCR assay. Plant Pathol. 57:332-337.
67. Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S. V., Machado, M. A., Toth, I., Salmond, G., and Foster, G. D. 2012. Top 10 plant pathogenic bacteria in molecular Plant Pathology. Mol. Plant. Pathol. 13:614-629.
68. Miličević-Marčić, S., Gartemann, K.-H., Frohwitter, J., Eichenlaub, R., Todorović, B., Rekanović, E., and Potočnik, I. 2012. Characterization of *Clavibacter michiganensis* subsp. *michiganensis* strains from recent outbreaks of bacterial wilt and canker in Serbia. Eur. J. Plant Pathol. 1-15.
69. Müller, J., Völksch, B., Eisbein, K., and Griesbach, E. 2000. Induction of resistance to bacterial pathogens in the pathosystem tomato/*C. michiganensis* subsp. *michiganensis* III. Characterization of the resistance induction in tomato cell cultures. Z. Pflanzenkrankh. Pflanzenschutz 107 561-573.
70. Nemeth, J., and Vuurde, J. W. L. v. 2006. Development of immunofluorescence colony staining (IFC) for detection of *Xanthomonas campestris* pv. *vesicatoria* and *Clavibacter michiganensis* subsp. *michiganensis* in tomato seeds. Seed Sci. Technol. 34:85-100.
71. Olivier, V., Baloch, A., Drouin, A., Audusseau, C., Paillard, S., and Soubelet, H. 2010. Internal methods comparison study and inter-laboratory study on *C. michiganensis* subsp. *michiganensis* in tomato seeds. EPPO Bull. 40:248-256.
72. Pine, T. S., Grogan, R. G., and Hewitt, W. B. 1955. Pathological anatomy of bacterial canker of young tomato plants. Phytopathology 45:267-271.
73. Poysa, V. 1993. Evaluation of tomato breeding lines resistant to bacterial canker. Can. J. Plant Pathol. 15:301-304.
74. Quesada-Ocampo, L. M., Landers, N. A., Lebeis, A. C., Fulbright, D. W., and Hausbeck, M. K. 2012. Genetic structure of *C. michiganensis* subsp. *michiganensis* populations in Michigan commercial tomato fields. Plant Dis. 96:788-796.
75. Ricker, M. D., and Riedel, R. M. 1993. Effect of secondary spread of *Clavibacter michiganensis* subsp. *michiganensis* on yield of northern processing tomatoes. Plant Dis. 77:364-366.
76. Roldan-Arjona, T., Perez-Espinosa, A., and Ruiz-Rubio, M. 1999. Tomatinase from *Fusarium oxysporum* f. sp. *lycopersici* defines a new class of saponinases. Mol. Plant-Microbe Interact. 12:852-861.
77. Sandbrink, J. M., Vanooijen, J. W., Purimahu, C. C., Vrieling, M., Verkerk, R., Zabel, P., and Lindhout, P. 1995. Localization of genes for bacterial canker resistance in *Lycopersicon peruvianum* using Rflps. Theor. Appl. Genet. 90:444-450.
78. Savidor, A., Chalupowicz, L., Teper, D., Gartemann, K.-H., Eichenlaub, R., Manulis-Sasson, S., Barash, I., and Sessa, G. 2014. *C. michiganensis* subsp. *michiganensis* Vatr1 and Vatr2 transcriptional regulators are required for virulence in tomato. Mol. Plant-Microbe Interact. 10:1035-1047.
79. Savidor, A., Teper, D., Gartemann, K. H., Eichenlaub, R., Chalupowicz, L., Manulis-Sasson, S., Barash, I., Tews, H., Mayer, K., Giannone, R. J., Hettich, R. L., and Sessa, G. 2012. The *C. michiganensis* subsp. *michiganensis*-tomato interactome reveals the perception of pathogen by the host and suggests mechanisms of infection. J. Proteome Res. 11:736-750.
80. Schaad, N. W., and Frederick, R. D. 2002. Real-time PCR and its application for rapid plant disease diagnostics. Can. J. Plant Pathol. 24:250-258.
81. Schaad, N. W., Jones, J. B., and Chun, W. 2001. Laboratory guide for identification of plant pathogenic bacteria. American Phytopathological Society, St. Paul, MN.
82. Sen, Y., Zhu, F., Vandenbroucke, H., van der Wolf, J., Visser, R., and van Heusden, A. 2012. Screening for new sources of resistance to *C. michiganensis* subsp. *michiganensis* (Cmm) in tomato. Euphytica 190:309-317.
83. Sharabani, G., Shtienberg, D., Borenstein, M., Shulhani, R., Lofthouse, M., Sofer, M., Chalupowicz, L., Barel, V., and Manulis-Sasson, S. 2013. Effects of plant age on disease development and virulence of *C. michiganensis* subsp. *michiganensis* on tomato. Plant Pathol. 62:1114-1122.
84. Smith, E. F. 1910. A new tomato disease of economic importance. Science 31:794-796.
85. Sotirova, V., and Achkova, Z. I. 1989. Resistance of tomatoes to *Corynebacterium michiganense* (Smith) Jensen. XII. Eucarpia Congress.
86. Sotirova, V., Bogatsevska, N., and Stamova, L. 1994. Sources of resistance to bacterial diseases in tomato wild species. Acta Hort. (ISHS) 376:353-360.
87. Stork, I., Gartemann, K. H., Burger, A., and Eichenlaub, R. 2008. A family of serine proteases of *C. michiganensis* subsp. *michiganensis*: chpC plays a role in colonization of the host plant tomato. Mol. Plant Pathol. 9:599-608.
88. Strider, D. L. 1969. Bacterial canker of tomato caused by *Corynebacterium michiganense*: A literature review and bibliography. N.C. Agric. Exp. Stn. Tech. Bull.
89. Tancos, M. A., Chalupowicz, L., Barash, I., Manulis-Sasson, S., and Smart, C. D. 2013. Tomato fruit and seed colonization by *C. michiganensis* subsp. *michiganensis* through external and internal routes. Appl. Environ. Microbiol. 79:6948-6957.
90. Thy, B. D. 1976. Inheritance of resistance to *Corynebacterium michiganense* in tomato. Phytopathology 66:1116-1119.
91. Thy, B. D. 1972. Pedigree of CmVF 232. Report of Tomato Genetics Cooperative 22.
92. Thy, B. D. 1971. Resistance to *Corynebacterium michiganense* measured in six *Lycopersicon* accessions. Phytopathology 61:972-974.
93. Utkhede, R., and Koch, C. 2004. Biological treatments to control bacterial canker of greenhouse tomatoes. BioControl 49:305-313.
94. van Heusden, A. W., Koornneef, M., Voorrips, R. E., Bruggemann, W., Pet, G., Vrieling van Ginkel, R., Chen, X., and Lindhout, P. 1999. Three QTLs from *Lycopersicon peruvianum* confer a high level of resistance to *Clavibacter michiganensis* ssp. *michiganensis*. Theor. Appl. Genet. 99:1068-1074.
95. van Loon, L. C., Geraats, B. P. J., and Linthorst, H. J. M. 2006. Ethylene as a modulator of disease resistance in plants. Trends Plant Sci. 11:184-191.
96. Vandenbult, R. W., Zevenhuizen, L. P. T. M., Cordewener, J. H. G., and Dons, J. J. M. 1991. Characterization of the extracellular polysaccharide produced by *Clavibacter michiganensis* subsp. *michiganensis*. Phytopathology 81:619-623.
97. Veena, M. S., and van Vuurde, J. W. L. 2002. Indirect immunofluorescence colony staining method for detecting bacterial pathogens of tomato. J. Microbiol. Methods 49:11-17.
98. Vu, B., Chen, M., Crawford, R. J., and Ivanova, E. P. 2009. Bacterial extracellular polysaccharides involved in biofilm formation. Molecules 14:2535-2554.
99. Vulkova, Z. V., and Sotirova, V. G. 1993. Study of the three-genome hybrid *Lycopersicon esculentum* Mill. - *L. chilense* Dun. - *L. peruvianum* var 'humifusum' Mill. and its use as a source for resistance. Theor. Appl. Genet. 87:337-342.
100. Wallis, F. M. 1977. Ultrastructural histopathology of tomato plants infected with *Corynebacterium michiganense*. Physiol. Plant Pathol. 11:333-342.
101. Wittmann, J., Eichenlaub, R., and Dreiseikelmann, B. 2010. The endolysins of bacteriophages CMP1 and CN77 are specific for the lysis of *Clavibacter michiganensis* strains. Microbiology 156:2366-2373.
102. Wolf, v. d. J. M., Zouwen, v. d. P. S., Ludeking, D. J. W., Hamelink, R., and Schenk, M. F. 2012. Onderzoeksverslag 'Distributie van *C. michiganensis* subsp. *michiganensis* in tomatenplanten'. Plant Res. Int. Rep. 448.
103. Xu, X. L., Miller, S. A., Baysal-Gurel, F., Gartemann, K. H., Eichenlaub, R., and Rajashekara, G. 2010. Bioluminescence imaging of *Clavibacter michiganensis* subsp. *michiganensis* infection of tomato seeds and plants. Appl. Environ. Microbiol. 76:3978-3988.
104. Yang, X. E., Long, X. X., Ni, W. Z., Ye, Z. Q., He, Z. L., Stoffella, P. J., and Calvert, D. V. 2002. Assessing copper thresholds for phytotoxicity and potential dietary toxicity in selected vegetable crops. J. Environ. Sci. Heal. B, Pesticides, food contaminants, and agricultural wastes 37:625-635.
105. Zaluga, J., Stragier, P., Baeyen, S., Haegeman, A., Van Vaerenbergh, J., Maes, M., and De Vos, P. 2014. Comparative genome analysis of pathogenic and non-pathogenic *Clavibacter* strains reveals adaptations to their lifestyle. BMC Genomics 15:1-14.
106. Zaluga, J., Van Vaerenbergh, J., Stragier, P., Maes, M., and De Vos, P. 2013. Genetic diversity of non-pathogenic *Clavibacter* strains isolated from tomato seeds. Syst. Appl. Microbiol. 36:426-435.

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