EPPO Datasheet: *Clavibacter michiganensis*

Last updated: 2022-01-27

IDENTITY

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Preferred name: *Clavibacter michiganensis*
Authority: (Smith) Davis et al.
Taxonomic position: Bacteria: Actinobacteria: Micrococcales: Microbacteriaceae
Other scientific names: *Bacterium michiganense* Smith, *Clavibacter michiganensis subsp. michiganensis* (Smith) Davis et al., *Corynebacterium michiganense pv. michiganense* (Smith) Dye & Kemp, *Corynebacterium michiganense pv. michiganense* (Smith) Jensen, *Corynebacterium michiganense subsp. michiganense* (Smith) Jensen, *Corynebacterium michiganense* (Smith) Jensen
Common names: bacterial canker of tomato, bird's eye of tomato fruits, vascular wilt of tomato
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EPPO Categorization: A2 list
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EU Categorization: RNQP ((EU) 2019/2072 Annex IV)
EPPO Code: CORBMI

Notes on taxonomy and nomenclature

For many years, the pathogen causing bacterial canker of tomato has been called *Clavibacter michiganensis* subsp. *michiganensis*. In the 2010s, the division of *Clavibacter michiganensis* into subspecies started to be reviewed, and subspecies were progressively moved to the species level, such as *C. sepedonicus, C. capsici* and *C. nebraskensi*s (Li *et al*., 2018; Nouioui *et al*., 2018). More recently, based on genomic and phylogenetic analysis, *C. michiganensis* subsp. *phaseoli, C. michiganensis* subsp. *californiensis*, and *C. michiganensis* subsp. *chilensis* were also elevated to species rank, leaving only one subspecies, *C. michiganensis* subsp. *michiganensis* (Arizala *et al*., 2022; Osdaghi *et al* ., 2020). As a consequence, this remaining subspecies should now be called *C. michiganensis*.

HOSTS

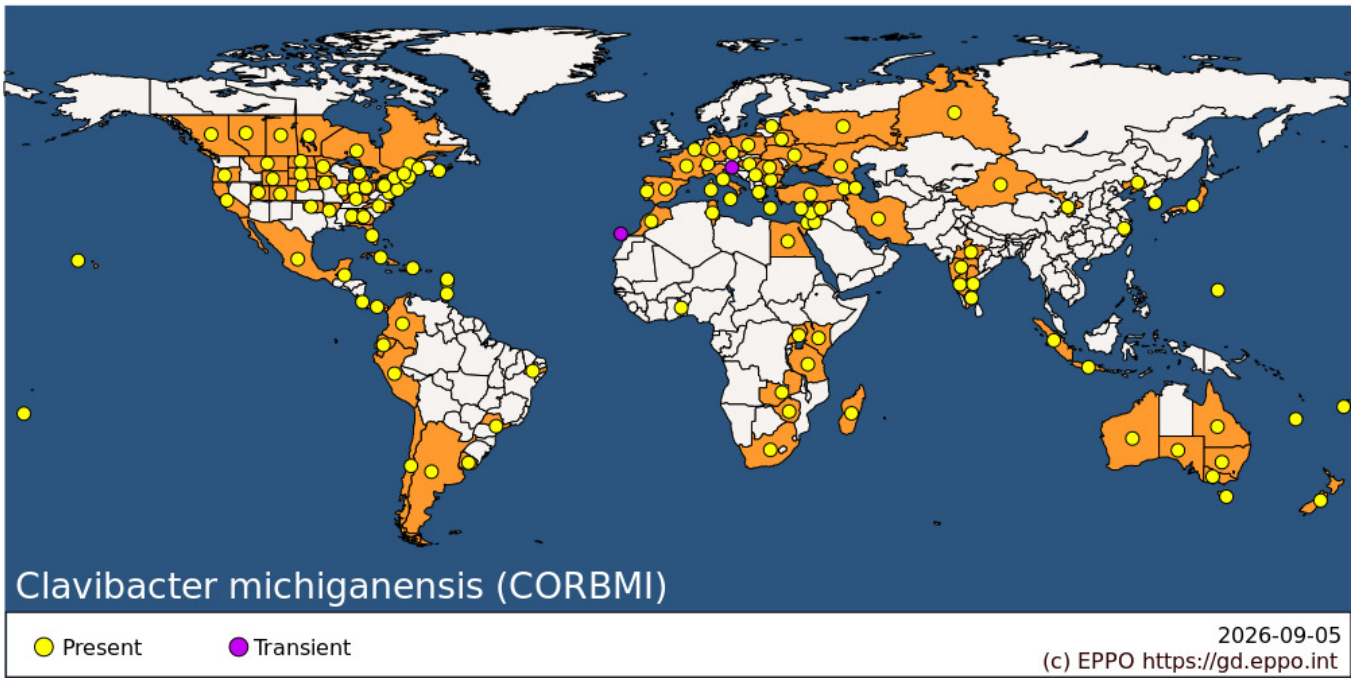
The only major host of economic importance is tomato (*Solanum lycopersicum*). Several strains which had been isolated from *Capsicum annuum* and *Capsicum frutescens* have been reclassified as *Clavibacter capsici* (Oh *et al*., 2016). Additionally, several weeds from the *Solanaceae* family have been shown to be susceptible to *C. michiganensis* under natural or experimental conditions (e.g. *Solanum douglasii*, *Solanum nigrum* and *Solanum triflorum*; Laj, 1976; Latin *et al*., 1995; Lewis Ivey & Miller, 2000; Nandi *et al*., 2018; Yim *et al*., 2012), and could be potential reservoirs of the pathogen. A number of solanaceous plants are susceptible after artificial inoculation (for details see Thyr *et al*., 1975; Yim *et al*., 2012), as well as other plant species including cucumber (*Cucumis sativus*), sunflower (*Helianthus* spp.) and watermelon (*Citrullus lanatus*). Additional monocotyledonous host plants reported susceptible after artificial inoculation, e.g. barley (*Hordeum vulgare*), maize (*Zea mays*), oat (*Avena sativa*) and wheat (*Triticum* spp.; Stamova & Sotirova, 1987) are considered doubtful pending confirmation.

Host list: *Solanum douglasii, Solanum lycopersicum, Solanum muricatum, Solanum nigrum, Solanum pectinatum, Solanum quitoense, Solanum triflorum, Solanum tuberosum*

GEOGRAPHICAL DISTRIBUTION

The bacterial canker of tomato was first identified in tomato greenhouses in Michigan (USA) in 1909, and is now widespread in Africa, Asia, Europe, North America, Oceania and South America. *S*ince the mid-twentieth century,

the intensification of the international tomato seed trade has caused *C. michiganensis* to spread within and between continents. It has also become widespread within a number of tomato producing countries of the EPPO region, but also in tomato producing countries outside the EPPO region.



EPPO Region: Armenia, Azerbaijan, Belarus, Belgium, Bulgaria, Cyprus, Czechia, France (mainland), Germany, Greece (mainland, Kriti), Hungary, Israel, Italy (mainland, Sardegna, Sicilia), Jordan, Latvia, Morocco, Poland, Portugal (mainland), Romania, Russian Federation (Central Russia, Southern Russia, Western Siberia), Serbia, Slovenia, Spain (mainland, Islas Canárias), Switzerland, Tunisia, Türkiye, Ukraine
Africa: Egypt, Kenya, Madagascar, Morocco, South Africa, Tanzania, United Republic of, Togo, Tunisia, Uganda, Zambia, Zimbabwe
Asia: China (Gansu, Liaoning, Xinjiang, Zhejiang), India (Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu), Indonesia (Java, Sumatra), Iran, Islamic Republic of, Israel, Japan (Honshu), Jordan, Korea, Republic of, Lebanon, Syrian Arab Republic
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Central America and Caribbean: Belize, Costa Rica, Cuba, Dominica, Dominican Republic, Grenada, Guadeloupe, Panama
South America: Argentina, Brazil (Pernambuco, Sao Paulo), Chile, Colombia, Ecuador, Peru, Uruguay
Oceania: Australia (New South Wales, Queensland, South Australia, Tasmania, Victoria, Western Australia), Fiji, Guam, New Caledonia, New Zealand, Tonga

BIOLOGY

Contaminated/infected tomato seeds and plants for planting are the primary source for *C. michiganensis* outbreaks. Contamination/infection levels as low as 1 in 10000 seeds can be enough to cause an epidemic. Plant debris containing the pathogen and alternative hosts can also play a role in disease outbreaks but are considered less important (De León *et al.*, 2011; EPPO, 2016a; Fatmi *et al.*, 1991; Moffett & Wood, 1984; Nandi *et al.*, 2018).

From the primary source the bacterium spreads locally mainly by water (rain splash, irrigation) and/or during cultural practices (e.g. trimming, chemical sprays). The bacterium can enter the plant tissue through hydathodes as well as different types of wounds on shoots and roots (Carlton *et al.*, 1998; Huang & Tu, 2001). Young plants have been shown to be more susceptible to *C. michiganensis* (Van Vaerenbergh & Chauveau, 1985). However, under

natural conditions, tomato plants are susceptible throughout their life (Nandi *et al.*, 2018). After infection, there is a latent period which can range from 7 days up to almost 3 months (EFSA, 2014; Gleason *et al.*, 1993). *C. michiganensis* can cause systemic infections, generally when the infection occurs at an early stage of plant development, or only a local infection, when the plant is infected later during its development (Sharabani *et al.*, 2013).

The bacterium colonizes and multiplies in the xylem vessels which allows it to spread rapidly through the plant. Inside the xylem *C. michiganensis* expresses a large set of virulence factors and enzymes leading, for example, to the degradation of the vascular tissue. Heavily infected vessels contain viscous granular deposits, tyloses and bacterial masses that block water transport which causes wilting of the plant. From the xylem, *C. michiganensis* can also enter the seed coat and endosperm leading to (new) seed infections (Nandi *et al.*, 2018). During later stages of infection, the stem can crack, leading to the exudation of droplets containing *C. michiganensis*, and allowing further spreading.

The optimum temperature for growth of *C. michiganensis* populations is 24-28°C. The bacterium is highly tolerant to desiccation and can survive on seeds and dried plant material for years. In dried soil, the survival time is shorter, but can last up to 7 or 8 months (EFSA, 2014).

DETECTION AND IDENTIFICATION

Symptoms

The EPPO Diagnostic Standard PM 7/42 for *C. michiganensis* gives a detailed description of the disease symptoms (EPPO, 2016a). Symptoms can be divided in those that are triggered during a systemic infection and those that can appear during a local infection.

In systemic infections early symptoms include the appearance of dull green, oily areas that desiccate and later turn brown. With temperatures of 25-30°C and strong evapotranspiration a reversible wilting of leaves occurs which, within a few days, will become irreversible. Entire leaves, and eventually the whole plant, will wilt and desiccate. Contaminated/infected seeds usually give rise to apparently healthy seedlings, symptoms only appearing as plants approach maturity. Fruits may fail to develop and fall, or ripen unevenly. They also often show external marbling and internal bleaching of vascular and surrounding tissue. Less frequently, fruits may show characteristic ‘bird's eye’ spots. Initially slightly raised and white, these spots develop light-brown roughened centres surrounded by a flat whitish halo. On cutting stems, petioles and peduncles, particularly at their junctions, a creamy-white, yellow or reddish-brown discoloration of vascular tissue and pith and cavities within the pith will be evident. These discolorations are only visible at advanced stages of the disease (EPPO, 2016a).

Local infections can cause a granular appearance of leaves, stems and calyces which is caused by the presence of raised or sunken white to pale orange blisters. Infections through hydathodes commonly lead to dark brown spots that are surrounded by a yellow to orange area at the edge of the leaf. At later stages the edges of these leaves can curl and wilt. Local infections can sometimes cause yellow streaks along the stem that might split open and form cankers (EPPO, 2016a).

Morphology

C. michiganensis is an aerobic, non-motile, Gram-positive and non-spore forming bacteria, with a curved rod shape (for details see Bradbury, 1986).

Isolation of the causal organism can be performed on e.g. yeast peptone glucose agar. On this medium the bacterium develops flat and semi-fluidal, round or irregular, yellow colonies in 3 to 4 days (EPPO, 2016a). White, pink, red and orange mutants may also occur (Hayward & Waterston, 1964).

Detection and inspection methods

Visual inspection will generally allow detection of symptoms during the growing season. However, plantlets are usually symptomless when they are traded, and symptoms are not visible on seeds. Plants for planting should be

inspected according to EPPO Standard PM 3/77 *Vegetable plants for planting under protected conditions – inspection of places of production* (EPPO, 2016b), whereas sampling of seeds for testing should follow EPPO PM 3/80 *Consignment inspection of seed of Solanum lycopersicum* (EPPO, 2021).

The EPPO Diagnostic Standard PM 7/42 for *C. michiganensis* provides thorough instructions on sampling, extracting, plating and diagnosing both symptomatic and symptomless plants. These include plating on both semi-selective and non-selective media and detection by immunofluorescence (IF) as well as PCR. Additionally, two procedures for detection of *C. michiganensis* in seeds are provided. The first procedure is based on plating seed extracts on different semi-selective media while the second procedure provides details on IF, PCR and selective enrichment protocols (EPPO, 2016a).

In addition, in recent years, a number of often TaqMan based qPCR protocols and a loop-mediated isothermal amplification assay were developed that can be used to confirm the identity of isolated bacteria suspected of being *C. michiganensis* and/or to specifically detect the bacterium in infected seeds and plant material (Dobhal *et al.*, 2019; Han *et al.*, 2018; Larrea-Sarmiento *et al.*, 2019; Ramachandran *et al.*, 2021; Thapa *et al.*, 2020).

PATHWAYS FOR MOVEMENT

Seed is the main long-distance pathway for movement of the pathogen. Cultivation measures contribute to local dispersion of the disease. Overhead irrigation, chemical sprays, handling during transport and wound inflicting actions such as clipping and pruning all favour the spread of *C. michiganensis*. The use of rotary mowers for clipping tomato plants has especially favoured disease dissemination (Carlton *et al.*, 1998; Huang & Tu, 2001; Sharabani *et al.*, 2013). Additionally, remaining contaminated crop debris can allow re-infection of the seedlings in the following season (EFSA, 2014).

PEST SIGNIFICANCE

Economic impact

Tomato is the world's most important vegetable crop with an annual production of over 180 million tonnes, representing a value of 93 billion USD (Food and agricultural organization, 2020). Since the first report of the disease in the USA in 1910, *C. michiganensis* has spread throughout the world and causes serious losses to both glasshouse and field tomato crops, either by killing the young plants or disfiguring the fruits making them unmarketable as fresh produce. Disease incidence in affected fields can be as high as 100% leading to severe yield losses. However, a high reduction in crop damage has been observed following the considerable efforts into preventing the introduction and dissemination of the pathogen, which involves the integral testing of seeds and plantlets (see Control). Compared to the damage in tomato, economic losses in pepper are limited (Baysal *et al.*, 2011; EFSA, 2014; Lamichhane *et al.*, 2011; Nandi *et al.*, 2018).

Control

Use of healthy seeds is the first and most important condition for controlling the disease. Seed lots should be laboratory tested for the presence of *C. michiganensis* and tomato seeds are often acid extracted to disinfect the seed surface (EPPO, 2021). A substantial reduction of infection can be achieved by chemical treatment of the seed (Dhanvantari, 1989). Once the disease has appeared in a crop, strict hygiene measures such as eradication of infected plants and isolation of infected rows can minimize yield loss. Prophylactic measures (destruction of crop residues, disinfection of structures and equipment) are essential to prevent infection in protected crops. At the moment there are no commercial tomato varieties which are fully resistant to *C. michiganensis* (Nandi *et al.*, 2018).

To prevent tomato seed and plant lots from being infected by *C. michiganensis* the 'Good Seed and Plant Practices' (GSPP) system was developed about a decade ago. GSPP provides standards for (hygiene) practices and participating companies are audited to ensure proper implementation (https://www.gspp.eu/).

Phytosanitary risk

Tomato is widely grown in glasshouses in the EPPO region, and the bacterium causes one of the most serious bacterial diseases of glasshouse tomatoes. For tomato field crops, the climatic conditions in southern Europe are favourable for disease development (EFSA, 2014). Disease outbreaks caused by *C. michiganensis* are sporadic but the impact of these outbreaks can be high. Since the pathogen is seed borne and contaminated/infected seed usually gives rise to apparently healthy seedlings, this facilitates introduction, establishment and spread. This combined with the fact that there are no curative treatments and no fully resistant commercial varieties available, make *C. michiganensis* a major threat for tomato cultivation (EFSA, 2014).

PHYTOSANITARY MEASURES

Since seeds are the main pathway for entry, appropriate measures could consist of treating the seeds (i.e. by means of an appropriate acid extraction method or an equivalent method) and testing seed lots according to EPPO Standards PM 7/42 (EPPO, 2021; EPPO, 2016a). Alternatively, seeds could be produced in a pest free area or in a pest free production site.

When not regulated as a quarantine pest, the EU Quality pest project recommended *C. michiganensis* for regulation as a RNQP, for propagation material (including seeds) of tomato (Picard *et al.*, 2018). An alternative measure was recommended, involving the absence of symptoms of disease caused by *C. michiganensis* observed in inspections, at appropriate times, during the complete cycle of vegetation of the plants at the site of production.

Additionally, good hygiene practices are important to prevent large disease outbreaks. The guidelines provided by GSPP describe measures that will help to limit the risks for *C. michiganensis* infections. These guidelines focus on managing risk factors, continuous monitoring and seed testing (https://www.gspp.eu/; EFSA, 2014).

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Datasheet history

This datasheet was first published in the EPPO Bulletin in 1982 and revised in the two editions of 'Quarantine Pests for Europe' in 1992 and 1997, as well as in 2022. It is now maintained in an electronic format in the EPPO Global Database. The sections on 'Identity', 'Hosts', and 'Geographical distribution' are automatically updated from the database. For other sections, the date of last revision is indicated on the right.

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