Research Article

Prevalence of (*Clavibacter michiganensis* subsp. *Michiganensis*) Causal Organism of Bacterial Canker in Weed Species in Tomato Fields of North West Pakistan

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Abstract | Bacterial canker is becoming one of the serious pests in vegetables in Northwest Pakistan. We investigated primary sources of inoculum for bacterial canker of tomato (BCT) caused by *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), in a set of 20 weeds commonly found in tomato fields of major tomato growing areas of Khyber Pakhtunkhwa (KP) province, Northwest Pakistan. Based on agroclimate, KP was divided into five zones as Northern KP, Southern KP, Eastern KP, Western KP and Central KP. A total of 100 collected weed samples were analyzed in laboratory for the presence of bacteria. The samples were crushed homogenized in 0.85% TBE buffer and platted on YDC (Yeast extract-Dextrose-$CaCO_3$) for bacterial growth. For identification of the characteristic bacterial colonies Cmm-specific primers CMM-5 and CMM-6 were used for amplification of 614bp product from *pat-1* gene of bacterial plasmid. The whole bacterial DNA was used as template, extracted with standard procedures using commercially available kit i.e. protinase-K (sigma). 27 out of 34 isolates were confirmed as Cmm having 614bp band. Black nightshade (*Solanum nigrum* L) was the only weed from which pathogen was consistently isolated. From Northern KP, a total of nine weeds including; *Solanum nigrum* L., *Solanum americanum* Mill., *Solanum Sarrachoides* Sedntner, *Amaranthus blitoides* S. Wats, *Amaranthus albus* L., *Lactuca serriola* L., *Amaranthus retroflexus* L., *Malva parviflora* L. and *Sisymbrium irio* L.were found to be possible sources of primary inoculum of the pathogen in tomato fields.

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Introduction

Tomato (*Solanum lycopersicum*), is one of the major cash crops for small-scale farmers in Northwest Pakistan. In spite of being cultivated on a large area, the average per hectare yield of tomato in Pakistan is approximately 10 ton/ha (FAOSTAT, 2015) which ranks the country far below the world's average (35 tons/ha).

Several factors contributes to this low yield and quality including abiotic and biotic stresses. Among biotic stresses, Bacterial canker of tomato (BCT) has become a major production constraint in the past couple of decadses in Pakistan. Bacterial canker of tomato, caused by bacterium *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) has a wide host range, from *Lycopersicon* species to some wild plants like *Solanum douglasii*, *S. nigrum* and *S. triflorum*. Furthermore, a number of solanaceous plants (Thyr et al., 1975); wheat, barley, rye, oats, sunflowers, watermelons and cucumbers (Stamova and Sotirova, 1987) are susceptible to the bacterium through artificial inoculation.

The disease not only affect tomato in tunnels but also in open fields, either by killing the young plants or disfiguring the fruits. Experiments carried out in France have shown a yield loss of 20-30% (Rat et al., 1991). Vasinauskiene (2002) reported that disease incidence reached from 10% to even 80% of tomato plants grown in some greenhouses.

In Pakistan, the etiology of the disease was not investigated. We infer, from surveys conducted among tomato growers in Northwest Pakistan, that the diseases is prevalent in the major tomato growing areas for the last 15 years. Lack of proper quarantine measures and free movement of infected seeds might be responsible for the introduction of the outbreak of the disease causing significant yield losses.

Although, healthy seed plays a vital role in the disease management (Thyr et al., 1973). The broad host range of Cmm highlights the importance of the weeds commonly grown in tomato fields. These weeds acts as an alternate host and become a source of primary inoculums for the next crop, causing major losses. The bacterium can survive inside the alternative host species which can serve as sources of infection (Strider, 1969). Leaf-surface populations on alternative hosts and on non-host plants can also play a vital role (Chang et al., 1992). In the field, the bacterium can spread through insect vector, rain splashes or mechanical contact etc. (Ricker and Riedel, 1993). Once the bacteria reach the target plant, pathogen use chemical or mechanical injuries, stomata and trichomes or hydathodes to invade the plants (Strider, 1969; Gleason et al., 1993; Carlton et al., 1998) and approach towards the xylem vessels (Leyns and De Cleene, 1983) where it can cause lysigenous cavities. Within 3-5 days localized leaf symptoms initiates (Basu, 1966; Layne, 1967) which turns to blister-like spots and tan-coloured lesions with white halos (i.e. bird's-eye spots) later on (Gleason et al., 1993; Carlton et al., 1998). Young plants are always more susceptible to the disease (Van Vaerenbergh and Chauveau, 1985).

Since, no research has been carried out to understand the epidemiology of the disease, we investigated the role of weeds as inoculum source for devising a better management strategy for bacterial canker disease in tomato. The research finding presented in this paper reports a comprehensive survey of weeds, for the presence of the bacterial canker pathogen, commonly found in tomato fields in Pakistan. We consistently identified a total of nine weeds as possible sources of primary inoculum of Cmm in tomato fields of Northwest of Pakistan.

Materials and Methods

Survey and sampling

In order to collect weeds plant samples from tomato field, a comprehensive survey was conducted in tomato-growing areas of Khyber Pakhtunkhwa (KP) and Federally Administrated Tribal Areas (FATA, agencies) from April to August, 2012. Khyber Pakhtunkhwa was divided into five Agro-ecological zones based upon weather, temperature, precipitation and geographic location etc. (Table 1) i.e. Northern KP; consisting of Malakand, Buneir, Mingora, Swat, Shangla, Dir upper, Dir lower, Chitral and Kalam, Southern KP consisting of D. I. Khan, Bannu, Hangu, Kohat, Karak, Lakki Marwat and Tank, Eastern KP consisting of Abbottabad, Naran, Kaghan, Mansehra, Haripur and Battagram and Western KP consisting of Khyber agency, Orakzai agency, Mohamand agency and North & South Waziristan and the most important Central KP consisting of Mardan, Nowshera, Peshawar, Swabi and Charsadda.

Table 1: *Agro-ecological zones of Khyber Pakhtunkhwa surveyed for weeds sampling during April to August, 2012.*

Agro-ecological zone	Areas surveyed
Northern KP	Malakand, Buner, Mingora, Swat, Shangla, Dir upper, Dir lower, Chitral, Kalam
Southern KP	D.I.Khan, Bannu, Hangu, Kohat, Karak, Lakki Marwat, Tank
Eastern KP	Abbottabad, Naran, Kaghan, Mansehra, Haripur, Battagram
Western KP	Khyber agency, Orakzai agency, Mohamand agency, North & South Waziristan
Central KP	Mardan, Nowshera, Peshawar, Swabi, Charsadda

A set of 100 samples of 20 weeds prevalent in tomato fields were collected from the infected fields in major tomato growing areas (representing all five agro ecological zones) of KP. Common weeds collected during the surveys included; black nightshade (*Solanum nigrum* L.), Prickly lettuce (*Lactuca serriola* L.), Prostrate pigweed (*Amaranthus blitoides* S. Wats.), American black nightshade (*Solanum americanum* Mill.),

Hairy nightshade (*Solanum Sarrachoides* Sedntner), Tumble pigweed (*Amaranthus albus* L.), Redroot pigweed (*Amaranthus retroflexus* L.), Cheeseweed (*Malva parviflora* L.), London rocket (*Sisymbrium irio* L.), Annual sow thistle (*Sonchus oleraceus* L.), Puncture vine (*Tribulus terrestris* L.), Johnsongrass (*Sorghum halepense* (L.) Pers.), Common lambs quarters (*Chenopodium album* L.), Nettle leaf goose foot (*Chenopodium murale* L.), Common purslane (*Portulaca oleracea* L.), Wright ground cherry (*Physalis acutifolia*), Field bindweed (*Convolvulus arvensis* L.), Large crabgrass (*Digitaria sanguinalis* (L.) Scop.), Annual bluegrass (*Poa annua* L.) and Yellow nutsedge (*Cyperus esculentus* L.).

Within each location, one to five fields were surveyed and two-three spots (consisting of 8-10 plants) were randomly chosen in each field. During sampling the whole plants were uprooted and collected. The samples were kept in paper bags, and tagged properly. Paper bags having samples were kept cool in icebox (containing ice-packets) and brought to the bacteriology lab, department of Plant Pathology, the University of Agriculture Peshawar. Samples yielding bacterial colonies having the morphology of Cmm were kept and those yielding no bacteria and yielding Gram negative bacteria or fungi were discarded.

Isolation of the pathogen from weeds

Plants were excised into small pieces, surface sterilized by dipping first in 20% Clorox solution for one minute and then rinsed twice with sterilized distilled water (SDW). These pieces were then homogenized in an equal amount of 0.85% TBE buffer. Homogenate was filtered through a muslin clothe and 100 µl of filtrate was platted directly on NA (Nutrient Agar) medium and the growing bacterial colonies were purified on YDC (Yeast extract-Dextrose-$CaCO_3$) medium. Inoculated petri plates were incubated at 29°C for 72 hours for bacterial growth (Wilson et al., 1967).

PCR confirmation of Cmm in weeds samples

Polymerase chain reaction (PCR) was used to confirm the identity of isolates. Cmm-specific primers CMM-5; 5'GCGAATAAGCCCATATCAA3' and CMM-6; 5'CGTCAGGAGGTCGCTAATA3' from plasmid-borne*pat-1* gene producing a 614bp amplification product, were used for this purpose (Ozdemir, 2005).

DNA extraction

For extracting DNA from Cmm isolates, the modified procedure of Li and De Boer (1995) was used. The whole bacterial DNA (to be used as template) was extracted with this procedure. Bacteria were grown overnight at 27°C in 5ml LB broth in a shaking incubator. A small amount (1.5ml) of liquid culture was transferred to Eppendorf tube, and pelleted by centrifugation at 7000 rpm for 10 minutes at 4°C. Supernatants were discarded and the pellets were frozen at -20°C for one hour and thawed at room temperature. Next, the pellets were treated with 100µl of cold acetone (-20°C) for 10 min, re-suspended in 0.5 ml of TE (10mM Tris hydrochloride, 1mM EDTA, pH 8.0) buffer, followed by addition of 50 µl sodium dodecyl sulfate (14%) and 10µl of 0.1% protinase-K (sigma). The mixture was then incubated for 1 hour at 55°C. An equal volume of 7.5M ammonium acetate was added to precipitate cell debris which were removed by centrifugation at 5000 rpm for 10 min. DNA in the supernatant was precipitated with cool isopropanol (-20°C) for 30 min. Then DNA (40-60ng) was pelleted by centrifugation at 13,000 rpm for 5 min and isopropanol was discarded. Pelleted DNA was washed (with 70% ethanol), vacuum dried and dissolved immediately in 100µl sterile distilled water.

PCR Amplification with species-specific primers

PCR for all 34 candidate isolates was performed in MJ mini the rmocycler (Bio-rad, USA). The 25 µl PCR reaction mixture contained 2.25 µl of 1x reaction buffer (20mM Tris-HCl, pH 8.4, 50mM KCl), 2 µl of 1.0 mM $MgCl_2$, 2.5 µl of 100 µM of each dNTP, 1 µl of 0.2 µM of each primer (forward and reverse), 1 µl of Taq DNA polymerase (Fermentas) and 3µl of (approx; 1ng) of template DNA. The PCR conditions used were: 94°C for 3 min followed by 30 reaction cycles of 94°C for 30s (denaturation), 55°C for 20s (primer annealing) and 72°C for 45s (primer extension). After the final reaction cycle the mixture was held at 72°C for 5 min and stored at 4°C. A negative control without template DNA was included in PCR amplification.

Gel electrophoresis

PCR product (25 µl aliquot per isolate per well) was electrophoresed through 2% agarose (0.667 gm agarose dissolved in 30 ml TBE buffer) gel. Agarose was dissolved in TBE buffer by heating in an oven for 2 minutes and after cooling down to 45-50°C, it was poured into gel electrophoresis apparatus tray and allowed to solidify at room temperature.

Table 2: *Isolation of Cmm (on YDC medium) from weeds commonly growing in commercial tomato fields of KP, Pakistan.*

S. No	Common name	Botanical name	Collection Districts				
			North KP*	South KP	East KP	Center KP	West KP
1	Black nightshade	*Solanum nigrum* L.	+	+	+	+	+
2	Prickly lettuce	*Lactuca serriola* L.	+	+	+	-	+
3	Prostrate pigweed	*Amaranthus blitoides* S. Wats.	+	-	+	+	+
4	American black nightshade	*Solanum americanum Mill.*	+	-	+	+	-
5	Hairy nightshade	*Solanum Sarrachoides Sedntner*	+	+	+	-	-
6	Tumble pigweed	*Amaranthus albus* L.	+	-	-	+	-
7	Redroot pigweed	*Amaranthus retroflexus* L.	+	-	+	-	-
8	Cheeseweed	*Malva parviflora* L.	+	-	+	-	-
9	London rocket	*Sisymbrium irio* L.	+	-	-	+	-
10	Annual sow thistle	*Sonchus oleraceus* L.	-	-	-	-	-
11	Puncture vine	*Tribulus terrestris* L.	-	-	-	-	-
12	Johnsongrass	*Sorghum halepense* (L) Pers.	-	-	-	-	-
13	Common lambs quarters	*Chenopodium album* L.	-	-	-	-	-
14	Nettle leaf goose foot	*Chenopodium murale* L.	-	-	-	-	-
15	Common purslane	*Portulaca oleracea* L.	-	-	-	-	-
16	Wright ground cherry	*Physalis acutifolia*	-	-	-	-	-
17	Field bindweed	*Convolvulus arvensis* L.	-	-	-	-	-
18	Large crabgrass	*Digitaria sanguinalis* (L.) Scop.	-	-	-	-	-
19	Annual bluegrass	*Poa annua* L.	-	-	-	-	-
20	Yellow nutsedge	*Cyperus esculentus* L.	-	-	-	-	-

+: *Cmm isolated/detected;* **-:** *Cmm not isolated/detected;* ***Northern KP:** *Malakand, Buner, Mingora, Shangla, swat, Dir upper, Dir lower, Chitral, Kalam;* **Southern KP:** *D.I. Khan, Bannu, Hangu, Kohat, Karak, Lakki Marwat, Tank ;* **Eastern KP :** *Abbottabad, Naran, Kaghan, Mansehra, Haripur, Battagram;* **Western KP:** *Khyber agency, Orakzai agency, Mohamand agency, North and South Waziristan;* **Central KP:** *Mardan, Nowshera, Peshawar, Swabi, Charsadda.*

After solidification, enough TBE buffer was added to cover the top of the gel. The PCR product (25 µl; after mixing with the blue tracking dye) of each isolate was loaded into the wells of the gel. 1Kb DNA ladder was used for size comparison. Electrophoresis was performed according to standard procedures (200 volts, 30 mints). The gel was stained in ethidium bromide (0.5µg/ml) solution for 15 minutes to visualize the amplified DNA and washed with sterile distilled water for 15 min. The ethidium bromide-stained bands were observed under UV light in UV tech machine (ESSENTIAL, D-55-20-M-Auto., UK) and the images were saved (Figure 1).

Results and Discussion

Twenty tomato weeds, occurring commonly in tomato fields from all the five agro-ecological zones were tested for isolation of the pathogen. Out of total 100 samples 52 produced bacterial colonies on NA medium. These 52 different colonies were then purified by sub-culturingon YDC medium. Gram staining and 3% KOH test, when performed for these isolates, 34 out of 52 proved to be Gram positive. The remaining 18 (Gram negative) were discarded, while the others were subjected to PCR for their genetic confirmation with Cmm-specific primers. 27 out of 34 isolates were confirmed to be Cmm as they successfully amplified 614bp band from plasmid-born *pat-1* gene (Figure 1).

Data (Table 2) showed that *Solanum nigrum* L (Black nightshade) was the only weed from which pathogen was consistently isolated (regardless of the collection zone). In case of *Amaranthus blitoides*, pathogen was recovered from all such weeds except those collected from Southern KP. Same way, *Lactuca serriola* weeds harbored the pathogen except those collected from central KP. However, pathogen was not recovered from *Sonchus oleraceus*, *Tribulus terrestris*, *Sorghum halepense*, *Chenopodium album*, *Chenopodium murale*, *Portulaca oleracea*, *Physalis acutifolia, Convolvulus ar-*

vensis, *Digitaria sanguinalis*, *Poa annua* and *Cyperus esculentus*.

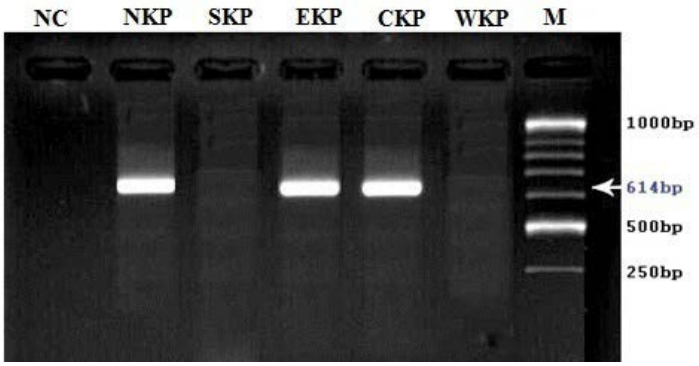

Figure 1: *Amplification of the Cmm-specific 614bp pat-1 gene fragment from collectedisolates of American black nightshade (Solanum americanum Mill.).*
M: *1kb Marker for size comparison;* **NKP:** *Northern zone of Khyber Pakhtunkhwa;* **SKP:** *Southern zone of Khyber Pakhtunkhwa;* **EKP:** *Eastern zone of Khyber Pakhtunkhwa;* **CKP:** *Central zone of Khyber Pakhtunkhwa;* **WKP:** *Western zone of Khyber Pakhtunkhwa;* **NC:** *Negative control.*

On the other hand in Northern KP, a total of nine weeds were found to be harboring the pathogen. These weeds included *Solanum nigrum*, *Solanum americanum*, *Solanum Sarrachoides*, *Amaranthus blitoides*, *Amaranthus albus*, *Lactuca serriola*, *Amaranthus retroflexus*, *Malva parviflora* and *Sisymbrium irio*. In case of Eastern KP, the pathogen was isolated from seven weeds i.e. *Solanum nigrum*, *Solanum americanum*, *Solanum Sarrachoides*, *Amaranthus blitoides*, *Lactuca serriola*, *Amaranthus retroflexus* and *Malva parviflora*. The five weeds were *Solanum nigrum*, *Solanum americanum*, *Amaranthus blitoides*, *Amaranthus albus* and *Sisymbrium irio*collected from Central KP had the pathogen. In Western and Southern KP, the pathogen was recovered from three weeds each i.e. *Solanum nigrum*, *Solanum Sarrachoides* (*Amaranthus blitoides* at Southern KP) and *Lactuca serriola*.

Some of the weeds having biology like tomato and producing compounds which provide a good microenvironment for the existence of the bacterium, can serve as alternate host for Cmm. Solanaceous weeds serve as asymptomatic hosts on which the pathogen can multiply during the course of a growing season (Holmstrom, 2015). In general the main host of Cmm which is of economic importance is tomato, but the pathogen has also been reported on other *Lycopersicon* spp. and on the wild plants like *Solanum douglasii*, *S. nigrum* and *S. triflorum*. Natural infections have also been found on *Capsicum annuum* and *C. frutescens* (Strider, 1969; Lai, 1976; Moffett and Wood, 1984; Latin et al., 1995; Lewis-Ivey and Miller, 2000; Yim et al., 2012), and several solanaceous weeds (e.g. *Solanum nigrum*, *S. douglasii* and *S. triflorum*) (Bradbury, 1986). Among other solanaceous plants, aubergine (*S. melongena*) is susceptible upon artificial inoculation (Thyr et al., 1975). In this study nine weeds in tomato fields including; *Solanum nigrum*, *Solanum americanum*, *Solanum Sarrachoides*, *Amaranthus blitoides*, *Amaranthus albus*, *Lactuca serriola*, *Amaranthus retroflexus*, *Malva parviflora* and *Sisymbrium irio* were found to serve as an alternate host for the pathogen however, the presence of symptomless pathogen supported the findings of Bradbury (1986), Holmstrom (2015) other scientists.

In the present study in some of the weeds, the pathogen was not detected in some particular area but in other are they do carry the bacteria. This phenomenon might be attributed to genetic makeup of the pathogen as well as the plant which have slight variations due to their geographical distribution which include all other factors e.g. weather, temperature, precipitation and topography etc. Andreote et al. (2010) also believed that specific bacterial functions are required for plant colonization, but also from the plant side specific features are needed, such as plant genotype (cultivar) and developmental stage. Plant growth stage was the most important factor influencing the composition of the bacterial communities. Bacterial inoculation of plants may lead to shifts in the composition of plant-associated bacterial communities (Andreote et al., 2006; Viebahn et al., 2005) and this on its turn may also lead to differences in plant metabolism. Cultivar effects (which is generally not studied in weeds) might having effect on bacterial survival. With the above justification our result are in line with the findings of Andreote et al. (2010). A particular weed cannot be attributed as host for the pathogen due the presence of the pathogen. It might be quite possible that the pathogen is instantly transferred during sampling or insect vector activity. The significance of these epiphytic populations is not fully understood, although they appear to contribute to infections through pruning wounds (Carlton et al., 1994).

Conclusion and Recommendation

From the present research it can be concluded that the naturally grown local weeds in tomato fields can serve as an alternate host for the pathogen and play

an important role of primary inoculum in disease development. To reduce the amount of primary inoculum, weeds inside and around tomato fields should be controlled thoroughly.

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Author's Contribution

Bibi, A. performed the experiment; Bibi, A. and Ahmad, M. wrote the first draft of the manuscripts. Hussain, S. designed the experimentation and Bibi, A. analysed the data. All authors read and approved the final manuscript.

References

Andreote, F.D., P.T. Lacava, C.S. Gai, W.L. Araújo, W. Maccheroni, L.S. Overbeek, J.D. Elsas and J.L. Azevedo. 2006. Model plants for studying the interaction between *Methylobacterium mesophilicum* and *Xylella fastidiosa*. Can J. Microbiol. 52: 419–426. https://doi.org/10.1139/w05-142

Andreote, F.D., U.N. Rocha, W.L. daAraújo, J.L. Azevedo and L.S. van-Overbeek. 2010. Effect of bacterial inoculation, plant genotype and developmental stage on root-associated and endophytic bacterial communities in potato (*Solanum tuberosum*). Antonie Van Leeuwenhoek. 97(4): 389–399. https://doi.org/10.1007/s10482-010-9421-9

Basu, P.K. 1966. Conditions for symptomatological differentiation of bacterial canker, spot, and speck on tomato seedlings. Canadian J. Plant Sci. 525–30. https://doi.org/10.4141/cjps66-088

Bradbury, J.F. 1986.Guide to plant pathogenic bacteria. CAB International, Wallingford, UK.

Carlton, W.M., E.J. Braun, M.L. Gleason. 1998. Ingress of *Clavibacter michiganensis* subsp. *michiganensis* into tomato leaves through hydathodes. *Phytopathol.* 88: 525–9. https://doi.org/10.1094/PHYTO.1998.88.6.525

Carlton, W.M., M.L. Gleason and E.J. Braun. 1994. Effects of pruning on tomato plants supporting epiphytic populations of *Clavibacter michiganensis* subsp. *michiganensis*. Plant Dis. 78:742–745. https://doi.org/10.1094/PD-78-0742

Chang, R.J., S.M. Ries, J.K. Pataky. 1992. Local sources of *Clavibacter michiganensis* subsp. *michiganensis* in the development of bacterial canker on tomatoes. *Phytopathol.* 82: 553–60. https://doi.org/10.1094/Phyto-82-553

FAOSTAT. 2015. FAO (Food and Agricultural Organization of the United Nations). Available online at: http://faostat.fao.org.

Gleason, M.L., R.D. Gitaitis, M.D. Ricker. 1993. Recent progress in understanding and controlling bacterial canker of tomato in eastern North America. Plant Dis.77: 1069–76. https://doi.org/10.1094/PD-77-1069

Holmstrom, K. 2015. Bacterial canker of tomato. Rutgers. New Jersey Agriculture experiment station. PP, 1-7.Available online at: http://nj-vegetable-crops-online-resources.rutgers.edu/wp-content/uploads/2015/06/Bacterial-Canker-Tomato.pdf. (Accessed 20 May 2015).

Lai, M. 1976. Bacterial canker of bell pepper caused by *Corynebacterium michiganense*. Plant. Dis. Rep. 60:339–342.

Latin, R., I. Tikhonova and K. Rane. 1995. First report of bacterial canker of pepper in Indiana. Plant. Dis. 79: 860. https://doi.org/10.1094/PD-79-0860E

Layne, R.E.C. 1967. Foliar trichomes and their importance as infection sites for *Corynebacterium michiganense* on tomato. *Phytopathol.* 57: 981–5.

Lewis-Ivey, M.L. and S.A. Miller. 2000. First report of bacterial canker of pepper in Ohio. Plant. Dis. 84:810–811. https://doi.org/10.1094/PDIS.2000.84.7.810C

Leyns, F. and M de Cleene. 1983. Histopathology of the bacteriosis caused by inoculation of *Corynebacteriummichiganense* and *Xanthomonascampestris*pv. *vesicatoria* in tomato stems. Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent. 48(3):663-670.

Li, X. and S.H. De-Boer. 1995. Selection of polymerase chain reaction primers from an RNA intergenic spacer region for specific detection of *Clavibactermichiganensis* subsp. *sepedonicus*. Phytopathol. 85:837-842. https://doi.org/10.1094/Phyto-85-837

Moffett, M.L. and B.A. Wood. 1984. Survival of *Corynebacterium michiganense* subsp. *michiganense* within host debris in soil. Aust. Plant. Pathol. 13:1–3. https://doi.org/10.1071/APP9840001

Ozdemir, Z. 2005. First Report of Fruit Infections of *Clavibacter michiganensis* subsp. *michiganensis* , on Processing Tomato in Turkey. Plant. Pathol. J. 2:143-145.

Rat, B., J. Poissonnier, M.J. Goisque and A. Burgaud. 1991. Le point sur le chancre bactérien. Fruit et Légumes. 86:38-40.

Ricker, M.D. and R.M. Riedel. 1993. Effect of secondary spread of *Clavibactermichiganensis* subsp. Michiganens is on yield of northern processing tomatoes. Plant Dis. 77(4): 364-366. https://doi.org/10.1094/PD-77-0364

Smith, A.F. 1994. The tomato in America: early history, culture, and cookery. Columbia, S.C, USA: University of South Carolina Press. ISBN 1-5700-3000-6.

Stamova, L. and V. Sotirova. 1987. Reaction of different crops to artificial inoculation with *Corynebacterium michiganense*. Arch. Phytopathol. Pflanzensc. 23:211-216. https://doi.org/10.1080/03235408709438079

Strider, D.L. 1969. Bacterial canker of tomato caused by *Corynebacterium michiganense*. A literature review and bibliography. Technical Bulletin No 193, North Carolina Agricultural Experiment Station, Raleigh, North Carolina, pp. 110.

Strider, D.L. 1969. Bacterial Canker of Tomato Caused by Corynebacterium michiganense: A Literature Review and Bibliography. Raleigh, NC, USA: North Carolina Agricultural Experiment Station.

Thyr, B.D., M.J. Samuel and P.G. Brown. 1975. New solanaceous host records for *Corynebacterium michiganense*. Plant. Dis. Rep.59:595-598.

Thyr, B.D., R.E. Webb, C.A. Jaworski and T.J. Ratcliffe. 1973. Tomato bacterial canker: control by seed treatment. Plant. Dis. Rep. 57: 974-977.

Van Vaerenbergh, J. and J.F. Chauveau. 1985. Host-plant inoculations for the detection of (latent) *Corynebacteriummichiganense* (E. F. Smith) Jensen. Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent. 50(3a): 973-995.

Vasinauskienë, M. 2002.Bacterial diseases of greenhouse-grown tomatoes. Biologiya. 1:29–31.

Viebahn, M., R. Doornbos, K. Wernars, L.C. Loon, E. Smit, P. Bakker. 2005. Ascomycete communities in the rhizosphere of field-grown wheat are not affected by introductions of genetically modified *Pseudomonas putida* WCS358r. Environ Microbiol. 7: 1775–1785. https://doi.org/10.1111/j.1462-2920.2005.00783.x

Wilson, E.E., P.M. Zeitoun and F.M. Fredrickson. 1967. Bacterial Phloem cankar: A new disearse of Persian walnut trees. Phytopathology. 57: 618-621.

Yim, K. O., H .I. Lee, J.H. Kim, S.D. Lee, J.H. Cho and J.S. Cha. 2012. Characterization of phenotypic variants of *Clavibacter michiganensis* subsp. *michiganensis* isolated from Capsicum annuum. Euro. J. Plant. Pathol. 133:559–575. https://doi.org/10.1007/s10658-011-9927-7