JARQ 25, 27–32 (1991)

Ecology and control of Tomato Bacterial Canker and Detection Methods of Its Pathogen

Takashi SHIRAKAWA, Tsugio SASAKI* and Katsumi OZAKI
Morioka Branch, National Research Institute of Vegetables, Ornamental Plants and Tea
(Shimokuriyagawa, Morioka, Iwate, 020-01 Japan)

Abstract
The present paper reviews results of series of the studies pertaining to ecology and control of bacterial canker of tomato caused by Clavibacter michiganensis subsp. michiganensis. The infection of this disease took place when a population density of the causal bacteria was more than 10^6 cfu/g of tomato leaves, while there was no disease under the density of less than 10^3-10^5 cfu/g. Occurrence of bacterial canker in tomato was severer in an open field than in a protected field from rainwater. It may be concluded that multiplication and dissemination of causal bacteria are augmented under a rainfall condition in an open field. This result suggests that the cultivation of tomato plants under plastic films to avoid rainfalls be one of the practical methods for controlling bacterial canker. The selective medium, SMCMM which was developed for isolating causal bacteria from plant materials or from soils, was useful for an ecological study of this disease, especially for checking a population density of causal bacteria. It was recognized that the ELISA method could be used for detection of C. m. ssp. michiganensis and for the rapid diagnosis of the disease.

Discipline: Plant disease
Additional key words: Clavibacter michiganensis subsp. michiganensis, ELISA, selective medium

Introduction

In 1958, occurrence of bacterial canker in tomato was reported in Hokkaido, Japan. Since then, the incidences of this disease have taken place in tomato plants cultivated mainly in open fields located in low-temperature areas of Japan such as Tohoku district. This is now one of the most important tomato diseases in the northern part of the country. It is informed that the causal bacteria of the disease, *Clavibacter michiganensis* subsp. *michiganensis,* is transmitted through tomato seeds and that infected plant debris and soils are relevant as inoculum sources[9,12]. However, details on the mechanism of infection from inoculum sources had not fully been identified yet.

Sasaki et al.[4–6] implemented a series of studies on multiplication of the causal bacteria in tomato plants under the two conditions; one was an open field and the other was a nursery bed. In those studies, a relevant mechanism of infection was examined. In addition, an attempt was made to establish an effective method for detecting and measuring pathogen of tomato bacterial canker. The results obtained will be summarized in the following sections.

Transmission of causal bacteria in a nursery bed

Although the transmission of *C. m.* ssp. *michiganensis* through seed was already confirmed[12], only limited information are available regarding the secondary transmission of this pathogen in a nursery bed. Sasaki[4] pursued the dissemination of causal bacteria of this disease originated from a contaminated mass of seeds, in which the relevant bacteria were isolated from 30% of those seeds. The bacterial population in the seedlings originated from that mass was 10^3–10^4 cfu/g of tomato shoots. The density of bacterial population on the normally grown seedlings adjacent the seedlings originated from the

* Present address: Kariwano, Nishisenboku, Senboku, Akita, 019-21 Japan

contaminated seeds was 10^3–10^4 cfu/g. In addition, it was observed that the causal bacteria were isolated from 75% of the seedlings which were grown near the artificially inoculated plants, and that the population density of the causal bacteria on those seedlings was 10^3–10^5 cfu/g. It may therefore be concluded that the causal bacteria were multiplied during the growth of the seedlings originated from the contaminated seeds and that such seedlings contribute to the secondary infection as inoculum sources.

Relationship between the population of *C. m.* ssp. *michiganensis* and the occurrence of tomato bacterial canker

Thyr[11] demonstrated that the tomato seedlings could be infected by *C. m.* ssp. *michiganensis* with only a few as five cells when the causal bacteria were directly inoculated into a xylem tissue. Strider[10] indicated that tomato plants could be infected by the stem-inoculation with only nine cells per m*l* of this causal pathogen. However, there had been no data indicatig a relationship between the occurrence of tomato bacterial canker and the population of its causal pathogen.

Sasaki[4] reported that in his artificial inoculation test, tomato plants were not infected, when the population size of the causal bacteria on the leaflets of tomato was less than 10^5 cfu/g of leaflets, while they were diseased at the level of more than 10^6 cfu/g. A similar result was obtained in an open field, where tomato plants were grown under a natural condition (Table 1). In two to six weeks after the first detection of causal bacteria, the plants got infected and the population size of *C. m.* ssp. *michiganensis* at that time was more than 10^6 cfu/g of leaflets. Based on these results, it may be concluded that when the population size of causal bacteria is less than 10^6 cfu/g, no disease may take place.

Protection of tomato bacterial canker by cultivation under plastic films

Sasaki et al.[5] compared the following two conditions in regard to the multiplication of causal bacteria: one was a protected condition from rainfall and the other was an open field. They further examined a mechanism of the effectiveness of the cover with a plastic film in protecting tomato plants from the disease. The results obtained are summarized as below.

The occurrence of bacterial canker under those two conditions is presented in Table 2. Almost all the tomato plants grown in the open field were infected by bacterial canker before the end of July both in 1984 and 1985, whereas all the plants grown under the plastic film were completely free from infection

Table 1. Colony population density of *C. m.* ssp. *michiganensis* on tomato leaflets in an open field

No. of field	Date of investigation (in 1984)							
	May 11	May 16	May 24	June 7	June 11	June 18	June 26	July 3
1	–[a]	–	1.1×10^5	2.1×10^6[b]	1.0×10^7	2.7×10^5	1.0×10^6	2.6×10^7
2	3.3×10^3	1.0×10^4	3.1×10^5	1.2×10^6	3.4×10^6	4.4×10^6	–	–
3	–	–	–	1.2×10^5	1.1×10^6	4.7×10^6	1.0×10^7	1.9×10^6
4	–	–	–	–	–	8.0×10^6	3.0×10^7	8.0×10^6
5	–	–	–	2.6×10^5	2.5×10^5	1.2×10^6	3.1×10^6	4.6×10^6
6	–	–	–	–	–	1.2×10^6	5.4×10^6	2.8×10^6
7	–	–	–	–	–	–	2.0×10^7	5.0×10^7
8	–	–	–	–	–	1.2×10^6	5.4×10^6	7.7×10^6
9	–	–	–	–	–	4.0×10^6	–	9.0×10^7
10	–	1.0×10^4	–	–	6.0×10^4	–	5.0×10^6	2.0×10^7
11	–	–	–	–	1.0×10^5	1.0×10^6	2.0×10^5	–

a): Not detected.

b): Underline; Outbreaks of bacterial canker of tomato.

Table 2. Changes of the rates with which the tomato bacterial canker disease occurrs under the rain-protected and open field conditions

Year	Treatment	Rate of occurrence (%)					
		July 4	July 11	July 18	July 24	July 31	Aug. 9
1984	Under a plastic film	0	0	0	0	0	0
	In an open field	17.5	77.5	100.0	100.0	100.0	100.0
		July 2	July 9	July 16	July 23	July 30	
1985	Under a plastic film	0	0	0	0	0	
	In an open field	2.6	38.0	78.2	100.0	100.0	

Table 3. Population density of *C. m.* ssp. *michiganensis* on tomato leaflets under a plastic film and in an open field

Year	Treatment	Sample no.	Population of *C.m.* ssp. *michiganensis* (cfu/g)					
			July 4	July 11	July 18	July 24	July 31	Aug. 9
1984	Under a plastic film	1	–[a)]	–	–	–	–	–
		2	–	8.0×10^4	–	–	–	–
		3	–	–	9.0×10^3	2.0×10^2	–	–
	In an open field	1	1.6×10^5	7.0×10^5	2.7×10^6	3.2×10^7	7.7×10^6	1.2×10^4
		2	2.7×10^5	3.0×10^5	3.0×10^5	1.5×10^7	9.0×10^3	–
		3	3.9×10^5	9.0×10^5	7.0×10^6	5.5×10^7	7.0×10^6	1.5×10^5
			July 2	July 9	July 16	July 23	July 30	
1985	Under a plastic film	1	–	–	–	–	–	
		2	2.0×10^4	–	4.6×10^3	–	–	
		3	–	1.0×10^2	–	–	–	
	In an open field	1	1.2×10^7	1.1×10^7	2.4×10^7	3.2×10^6	1.7×10^5	
		2	4.7×10^6	6.7×10^6	1.3×10^7	1.6×10^5	2.6×10^5	
		3	3.5×10^5	1.9×10^7	4.2×10^7	3.4×10^6	3.0×10^5	

a): Not detected.

throughout the period of investigation. The population densities of causal bacteria on the leaflets of tomato are shown in Table 3. As clearly indicated in this table, the causal bacteria isolated were very few in the tomato plants under the plastic film, while they were 10^5–10^7 cfu/g under the open field condition. This result indicates that the multiplication rate of causal bacteria is higher in an open field than under a condition covered by a plastic film. With the purpose of identifying a mechanism of the effectiveness of the cover with a plastic film, the relationship between the secondary transmission of the *C. m.* ssp. *michiganensis* and the rainfall was critically examined. Each investigation showed that the raindrops falling from the infected tomato plants contained about 10^6 cfu/m*l* of the causal bacteria, and that the water on the mulching film also contained causal bacteria with a density of 10^5–10^7 cfu/m*l*. From these results, it is concluded that the multiplication of *C. m.* ssp. *michiganensis* on tomato leaves and the secondary infection of this disease are augmented by rainfalls. It is therefore concluded that cultivation of tomato plants under the cover of a plastic film would be one of the effective methods for controlling the bacterial canker of tomato.

An improved method for detecting *C. m.* ssp. *michiganensis*

Regarding the media for detecting and measuring *C. m.* ssp. *michiganensis*, D_2 medium produced by Kado et al.[3] has been used, particularly in the case where the causal bacteria are associated with plant seeds and tissues. However, when D_2 medium used for detecting *C. m.* ssp. *michiganensis* from soils, the detection is rather difficult since many colonies

of other microorganisms are also produced. In order to resolve this problem, selective medium for detecting *C. m.* ssp. *michiganensis* (SMCMM) was produced by the authors[6], modifying CNS medium for *C. m.* ssp. *nebraskensis* developed by Gross et al.[2]. The composition of the medium is as follows: peptone; 5 g, yeast extract; 3 g, K_2HPO_4; 2 g, KH_2PO_4; 0.5 g, $MgSO_4 \cdot 7H_2O$; 0.25 g, glycerol; 20 g, LiCl; 5 g, agar; 15 g in 1*l* of distilled water. After autoclaving at 121°C for 15 min, it is cooled to 50°C, and then the following ingredients are added: $K_2Cr_3O_7$; 80 mg, NaN_3; 2 mg, nalidixic acid; 20 mg, cycroheximide; 40 mg, and 70% tetraisophthalonitrate (TPN); 3 mg. Nalidixic acid is dissolved in 0.1N NaOH solution.

With the above medium for detection, it was observed that the colonies of *C. m.* ssp. *michiganensis* on SMCMM came out in 3 to 4 days after plating when they were incubated at 25°C (Plate 1 & Table 4). The emerging colonies were circular, smooth, convex, and bright yellow with entire margin. On D_2 medium, they became to be visible in 4 to 5 days. The efficiency of colony formation on SMCMM for *C. m.* ssp. *michiganensis* was 1.2 to 4 times as high as that on the D_2 medium. When *C. m.* ssp. *michiganensis* was isolated from the infected plant materials, SMCMM was more effective than D_2 and CNS[2] medium in producing colonies. In detecting *C. m.* ssp. *michiganensis* from the artificially infested soils, a rate of recovery with SMCMM medium was the same with CNS medium and higher than that with D_2 medium. The colonies of *C. m.* ssp. *michiganensis* could be distinguished from other bacterial colonies with their morphological characteristics. Based on the above results, it may be concluded that SMCMM medium is more useful than the D_2 and CNS mediums for the study on ecology of bacterial canker of tomato, especially on population density of the pathogen. In addition to SMCMM, Fatmi et al. developed SCM medium to isolate *C. m.* ssp. *michiganensis* from the seed of tomato[1].

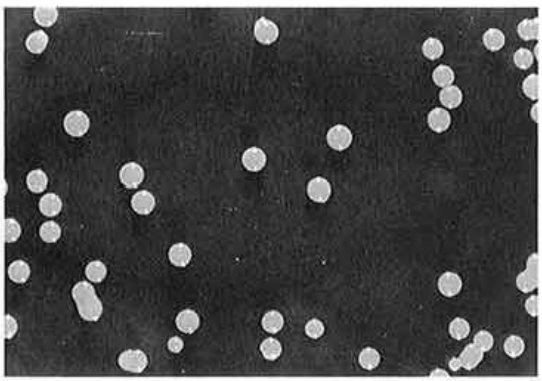

Plate 1. Colonies of *C. m.* ssp. *michiganensis* of a selective medium (SMCMM)

In a separate series of the study, an antiserum to *C. m.* ssp. *michiganensis* was produced, and the detection and the measurement of this pathogen by the ELISA method were examined (Fig. 1)[8]. The results obtained showed that a high reaction took place

Table 4. Isolation of *C. m.* ssp. *michiganensis* from the artificially inoculated tomato leaves and the artificially infested tomato rhizosphere soils with selective media, SMCMM

Sample	Medium	*C. m.* ssp. *michiganensis*		Other bacteria	
		No. of colonies[a]	Colony forming efficiency[b]	No. of colonies[a]	Colony forming efficiency[b]
Infected leaves	SMCMM	9.43×10^7	85.0	4.50×10^5	4.1
	CNS	6.57×10^7	59.0	3.75×10^5	3.4
	D_2	4.28×10^7	38.6	7.75×10^5	7.2
	NA	1.11×10^8	100.0	1.09×10^7	100
Infested soils	SMCMM	1.40×10^6	183	4.13×10^5	2.7
	CNS	1.50×10^6	196	5.25×10^5	3.4
	D_2	0	0	1.81×10^6	12.0
	NA	7.65×10^5	100	1.51×10^7	100

a): No. of colonies/g of fresh leaflets or soils.

b): Colony forming efficiency $= \frac{\text{No. of colonies on a selective medium}}{\text{No. of colonies on NA medium}} \times 100.$

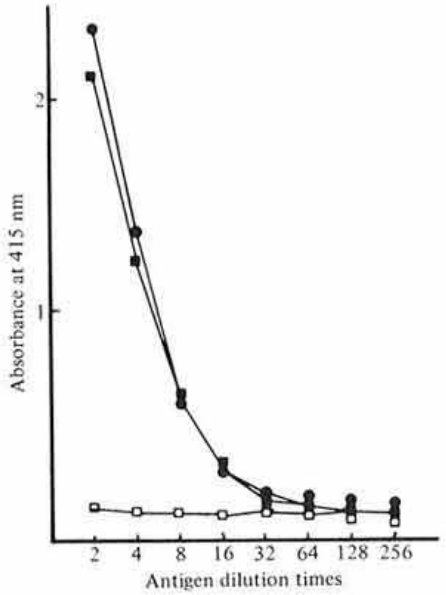

Fig. 1. ELISA values for inoculated and non-inoculated tomato leaves
●: Pure culture of *C. m.* ssp. *michiganensis* isolate YM 7709, ■: Tomato leaves inoculated with YM 7709, □: Tomato leaves without inoculation.

only on *C. m.* ssp. *michiganensis,* which could be detected over 1 × 10^5 cfu/m*l* level. However, since the ELISA values varied with the isolates of *C. m.* ssp. *michiganensis,* the population density of *C. m.* ssp. *michiganensis* could not be specified by the ELISA method. And they could be isolated at the level of over 10^6 cfu/g of tomato leaflets. The results of those experiments indicate that the ELISA method could be used for detecting *C. m.* ssp. *michiganensis.* On the basis of the relationship observed as above between the pathogen population density on tomato leaflets and the outbreaks of tomato bacterial canker, the ELISA method could be used for the rapid diagnosis of tomato bacterial canker. It has been recognized so far that the lowest level of pathogen in the detection with a dilution plate method with a selective medium is of the order of 10^2–10^3 cfu/g of the sample. Taking into account the comparative advantage of the above two methods, it may be said that the dilution plate method with a selective medium would be more sensitive than the ELISA method. However, if an experiment is undertaken to detect phytopathogenic bacteria from soils, it might be very difficult to distinguish colonies of the relevant bacteria from those of other microorganisms. Hence, a more suitable method for separation is required in the ecological studies on phytopathogenic bacteria. At this moment, therefore, it is suggested that an adequate method for detecting *C. m.* ssp. *michiganensis* be to employ a combined use of the following two means: i.e., the dilution plate method with SMCMM medium or SCM medium, and the ELISA method.

References

1) Fatmi, M. & Schaad, N. W. (1988): Semiselective agar medium for isolation of *Clavibacter michiganense* subsp. *michiganense* from tomato seed. *Phytopathology,* **78**, 121–126.
2) Gross, D. C. & Vidaer, A. K. (1979): A selective medium for isolation of *Corynebacterium netraskense* from soil and plant parts. *Phytopathology,* **69**, 82–87.
3) Kado, C. I. & Heslett, M. G. (1970): Selective media for isolation of *Agrobacterium, Corynebacterium, Erwinia, Pseudomonas* and *Xanthomonas. Phytopathology,* **60**, 969–976.
4) Sasaki, T. (1987): Ecological studies on bacterial canker of tomato. 3. Dissemination of causal bacteria from contaminated seeds and seedlings in the nursery bed. *Ann. Rep. Pl. Prot. North Jpn.,* **38**, 40–42 [In Japanese with English summary].
5) Sasaki, T. & Umekawa, M. (1986): Ecological studies on bacterial canker of tomato. 1. Relation between the population growth of pathogenic bacteria on the leaves and the occurrence of bacterial canker of tomato. *Ann. Rep. Pl. Prot. North Jpn.,* **37**, 65–67 [In Japanese with English summary].
6) Sasaki, T., Umekawa, M. & Honda, Y, (1986): Ecological studies on bacterial canker of tomato. 2. Development of bacterial canker of tomato under plastic roof and in open-field. *Ann. Rep. Pl. Prot. North Jpn.,* **37**, 68–70 [In Japanese with English summary].
7) Shirakawa, T. & Sasaki, T. (1988): A selective medium for isolation of *Corynebacterium michiganense* pv. *michiganense*, the pathogen of tomato bacterial canker disease. *Ann. Phytopath. Soc. Jpn.,* **54**, 540–543 [In Japanese with English summary].
8) Shirakawa, T. & Sasaki, T. (1990): Serological detection of *Clavibacter michiganensis* subsp. *michiganensis,* the pathogen of tomato bacterial canker, by enzyme linked immunosolvant assay. *Bull. Nat. Res. Inst. Veg. Ornam. Plants & Tea,* **C1**, 75–83 [In Japanese with English summary].
9) Strider, D. L. (1969): Bacterial canker of tomato caused by *Corynebacterium michiganense*: A literature review and bibliography. *North Carolina Agr. Exp. Sta. Tech. Bull.,* **193**, 1–110.

10) Strider, D. L. (1970): Tomato seedling inoculations with *Corynebacterium michiganense*. *Plant Dis. Reptr.*, **54**, 36–39.
11) Thyr, S. D. (1968): Bacterial canker of tomato: Inoculation level needed for infection. *Plant Dis. Reptr.*, **52**, 741–743.
12) Uematsu, T., Fujii, H. & Ohata, K. (1977): Relationship between inoculation methods and seed infection with tomato canker bacteria (*Corynebacterium michiganense*). *Ann. Phytopath. Soc. Jpn.*, **43**, 412–418 [In Japanese with English summary].

(Received for publication Sept. 14, 1990)