

Distribution of *Clavibacter michiganensis* subsp. *michiganensis* in Various Tomato Production Centers in Sumatra and Java

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Bacterial canker, caused by *Clavibacter michiganensis* subsp. *michiganensis* is a newly introduced disease of tomatoes in Indonesia. Its existence was first officially reported in 2004. The objective of this research was to monitor the existence of *C. michiganensis* subsp. *michiganensis* in various tomato production centers in Sumatra and Java. Tomato samples showing symptoms of *C. michiganensis* subsp. *michiganensis* infection were collected from various tomato production centers in Sumatra and Java and the causal agents were isolated from these samples. Based on the occurrences of typical symptoms of *C. michiganensis* subsp. *michiganensis* infection in tomato, the incidence of suspected *C. michiganensis* subsp. *michiganensis* infection ranged from 1-20%. From a total of 74 tomato plants sampled, 24 bacterial isolates exhibiting similar colony morphology to *C. michiganensis* subsp. *michiganensis* were obtained. After various physiological, hypersensitive response, and pathogenicity tests, 18 isolates derived from 14 tomato production centers in eight provinces in Sumatra and Java were identified as *C. michiganensis* subsp. *michiganensis*. Although the incidence was still low, results of these experiments further indicated that *C. michiganensis* subsp. *michiganensis* existed in Indonesia and had spread in a number of tomato production centers in Sumatra and Java. Positive results of *C. michiganensis* subsp. *michiganensis* identification from suspected tomato samples should be a warning sign for all tomato production stakeholders in Indonesia, especially those responsible in regulating the seed trade, importation, and plant quarantine.

Key words: bacterial canker, new disease in tomato, *Clavibacter michiganensis* subsp. *michiganensis*, isolate identification, field survey

Clavibacter michiganensis subsp. *michiganensis*, which causes bacterial canker disease of tomato, is a seed-transmitted bacterial pathogen. Bacterial canker has caused serious damage in tomato plants (Hayward and Waterston 1964). Yield reduction due to *C. michiganensis* subsp. *michiganensis* infection in tomatoes could be as high as 80% (Chang and Pataki 1992; Vasinauskienė 2002). Growth of *C. michiganensis* subsp. *michiganensis* and development of the disease were optimal in hot weather with a temperature of 26-28°C (Hayward and Waterston 1964).

Bacterial canker is a new disease in Indonesia since this country was reported free from this disease up to 2002. The detection of *C. michiganensis* subsp. *michiganensis* among commercially traded tomato seeds in Indonesia was first reported by Anwar *et al.* (2004a,b). There is a possibility that this pathogen has spread to a number of tomato production centers in Indonesia.

C. michiganensis subsp. *michiganensis* could have been introduced into production centers through infected seeds. It could quickly spread among tomato plants through various means and large scale infection of *C. michiganensis* subsp. *michiganensis* in tomato crops could take place over only a few seasons. It would be difficult to eradicate *C. michiganensis* subsp. *michiganensis* once the pathogen has been introduced and established in certain areas (Ark 1994; Fatmi and Schaad 2002). Therefore, monitoring of the existence of this pathogen and how wide it has spread in the field is a necessary step in preventing further spreading of bacterial canker. Direct collection of suspected *C. michiganensis* subsp. *michiganensis* from infected field grown tomato

followed by analysis using standard laboratory procedures for *C. michiganensis* subsp. *michiganensis* identification needs to be conducted.

The aim of this research was to monitor the existence of *C. michiganensis* subsp. *michiganensis* in various tomato production centers in Sumatra and Java. The specific objectives of this research were (i) to collect samples of tomato fruits and plants exhibiting symptoms of *C. michiganensis* subsp. *michiganensis* infection, (ii) to estimate disease incidences in the field based on symptoms of *C. michiganensis* subsp. *michiganensis* infection, (iii) to identify the presence of *C. michiganensis* subsp. *michiganensis* among collected tomato samples, and (iv) to determine the distribution of *C. michiganensis* subsp. *michiganensis* in various tomato production centres in Sumatra and Java.

MATERIALS AND METHODS

Collection of *C. michiganensis* subsp. *michiganensis* Infected Tomato Samples. Samples of *C. michiganensis* subsp. *michiganensis* from infected tomato plant tissues and fruits were collected up to July 2006. The collected samples consisted of leaves, stems, and fruits showing various symptoms associated with *C. michiganensis* subsp. *michiganensis* infection. Samples were collected using stratified-purposive-random-sampling from various major tomato production centers in Sumatra and Java. See results for the locations of tomato sample collection.

Collected plant materials were taken from each location and brought back directly or sent by express mail service (Titipan Kilat-TIKI) from the location to Padang, West Sumatra. The isolation of bacterial pathogens from tomato

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samples was conducted at the Bacteriology Lab, Phytopathology Department, Faculty of Agriculture, Andalas University, West Sumatra.

The incidence of suspected *C. michiganensis* subsp. *michiganensis* infection was evaluated by direct field observation in each location. The number of tomato plants showing symptoms of *C. michiganensis* subsp. *michiganensis* infection was recorded and percentages of symptom occurrences among tomato plantations were calculated.

Isolation of Suspected *C. michiganensis* subsp. *michiganensis* from Tomato Samples. Collected tomato samples (5 g) were dipped for 15 min in 15 ml of phosphate buffer tris (PBT) at 4°C and homogenized using a mortar and pestle. After centrifugation at 1 844 x g for 5 min, the supernatant plant extract was transferred into sterile micro-centrifuge tube and labeled as undiluted stock. Serial dilution of 10⁻¹ and 10⁻² were made from each of the undiluted stock using sterile PBT. Subsequently, each of the undiluted and diluted extracts (100 µl) was plated twice onto nutrient agar (NA) medium and the plates were incubated at 23-27°C.

Occurrences of bacterial colonies on the NA medium were evaluated 2 weeks after plating. All bacterial colonies showing characters similar to that of a reference *C. michiganensis* subsp. *michiganensis* colony were transferred onto YDC medium. Selected bacterial colonies growing on YDC medium showing similar characteristics of reference *C. michiganensis* subsp. *michiganensis* colony (yellow, wet, and mucoid) were isolated and evaluated for various physiological characters. Suspensions of *C. michiganensis* subsp. *michiganensis* isolate 542 (Anwar *et al.* 2004a; Anwar *et al.* 2005) at 10², 10³, and 10⁴ cfu ml⁻¹ were plated on both NA and YDC medium and used as the control.

Identification of Suspected Bacterial Isolates. Suspected *C. michiganensis* subsp. *michiganensis* colonies identified

from YDC medium were subjected to the Gram reaction (Suslow *et al.* 1982; Fatmi and Schaad 1988; Kritzman 1991), the production of fluorescent pigment, pectinase activity, oxidation, and nitrate reduction tests, respectively. The suspected isolates were also grown on TTC medium and tested for their tolerance against 6% NaCl. *C. michiganensis* subsp. *michiganensis* isolate 542 was also subjected to the same tests and used as the control.

After a series of physiological tests, bacterial isolates physiologically identified as similar to *C. michiganensis* subsp. *michiganensis* were subjected to the hypersensitive reaction (HR) test using leaves of *Nicotiana tabacum* and *Mirabilis jalapa* (Gitaitis 1990; Alarcon *et al.* 1998) and tested for their pathogenicity using tomato seedlings. The HR tests were conducted by injecting tested bacterial isolates into the leaves of *N. tabacum* and *M. jalapa* using 1 ml needle syringe (Alarcon *et al.* 1998; Anwar *et al.* 2005). The occurrence of necrotic lesions on injected leaves after 24 h indicated a positive result for the HR test.

Pathogenicity tests were conducted using four-week-old seedlings of *C. michiganensis* subsp. *michiganensis* susceptible-tomato cv. Marta. Inoculation of the seedlings with the tested bacterial isolates was conducted by cutting their epicotyls at 1 cm above their cotyledons using scissors. The scissors had previously been dipped in a suspension of the tested bacteria (10⁷ cfu ml⁻¹). Inoculated seedlings were covered with a transparent plastic bag to maintain humidity and incubated for 48 h in a glasshouse. Occurrences of typical symptoms associated with *C. michiganensis* subsp. *michiganensis* infection such as wilting of the cotyledons, tissue discoloration, and the wilting or death of seedlings were recorded and used to determine pathogenicity of the tested isolates against tomato plants.

Table 1 Location of collected tomato samples, observed symptoms of collected samples, disease incidence and number of bacterial isolates positively identified as *Clavibacter michiganensis* subsp. *michiganensis* from various tomato production centers in Sumatra and Java

Province: location, district	Observed symptoms on collected tomato samples	Disease inci-dence (%)*	No. of Bacterial isolates**
North Sumatera			
Peceran, Berastagi/Karo	Dwarf, blackened tomato stem	2-10	4
West Sumatera			
Danau Kembar, Solok	Bird's eye spot symptom	1-3	2
Lembah Gumanti, Solok	Split stem secretion	0.8	1
Tanjung Baru, Tanah Datar	Wilting leaves and necrosis at leaf perimeters	1-4	1
Baso, Agam	Wilting leaves and necrosis at leaf perimeters	2-11	1
Banuhampu, Agam	Stem necrosis (tissue discoloration)	2-10	1
IV Angkek, Agam	Wilting leaves and necrosis at leaf perimeters	1-2	1
Bengkulu			
Selupu Rejang, Rejang Lebong	Wilting leaves and necrosis at leaf perimeters, stem necrosis	0.8	1
Talang Rimbo, Kepahiang	Dwarf, blackened tomato stem	0.8	1
West Java			
Cipanas, Cianjur	Wilting leaves and necrosis at leaf perimeters	1-5	2
Pacet, Cianjur	Split stem secretion	1-7	1
Central Java			
Pejawanan, Banjarnegara	Stem necrosis, wilting leaves, and necrosis at leaf perimeters	2-20	3
Wanayasa, Banjarnegara	Split stem secretion and showing pith discoloration	5-10	2
East Java			
Matus, Kediri	Wilting leaves and necrosis	2-4	-
Kepung, Kediri	Split stem secretion and showing pith discoloration	2	2
Pujon, Malang	Stem necrosis, wilting leaves, and necrosis at leaf perimeters	3-20	1
Ngantung, Malang	Stem necrosis	5-12	-

*Disease incidences were calculated based on number of suspected symptoms observed in the field, **Identification of suspected bacterial isolates as *Cmm* was conducted based on morphological and physiological characteristics of the isolates.



Fig 1 Examples of tomato showing typical symptoms of suspected *Clavibacter michiganensis* subsp. *michiganensis* infection observed in the field. a, tomato fruit showing bird's eye spot symptoms (arrow) from Danau Kembar, Solok, West Sumatera and tomato plants showing; b, split stem secretion and showing pith discoloration (arrow) from Lembah Gumanti, Solok, West Sumatera; c, wilting leaves and necrosis at the leaf perimeters from Matus, Kediri, East Java; d, blackened stem, wilting leaves and necrosis at the leaf perimeters from Wanayasa, Banjarnegara, Central Java; and e, stem necrosis (tissue discoloration) from Cipanas, Cianjur, West Java.

RESULTS

Distribution of *C. michiganensis* subsp. *michiganensis* in Various Tomato Production Centers. Surveys conducted in various locations in Java and Sumatra (Table 1) led to the collection of 74 tomato samples exhibiting symptoms associated with *C. michiganensis* subsp. *michiganensis* infection. The samples consisted of leaves or stems (72 samples) and fruits exhibiting bird's-eye-spot symptoms (2 samples). Various symptoms exhibited by tomato

plants from a number of tomato production centers are presented in Table 1. Representative symptom exhibited by tomato plants and a fruit corrected from Solok, Kediri, Banjarnegara, and Cianjur are presented in Fig 1.

Incidence of Suspected *C. michiganensis* subsp. *michiganensis* Infection in the Field. Interviews with local farmers indicated that most of them did not recognize the symptoms of *C. michiganensis* subsp. *michiganensis* infection in tomatoes. Surveys conducted based on the occurrence of typical symptoms of *C. michiganensis*

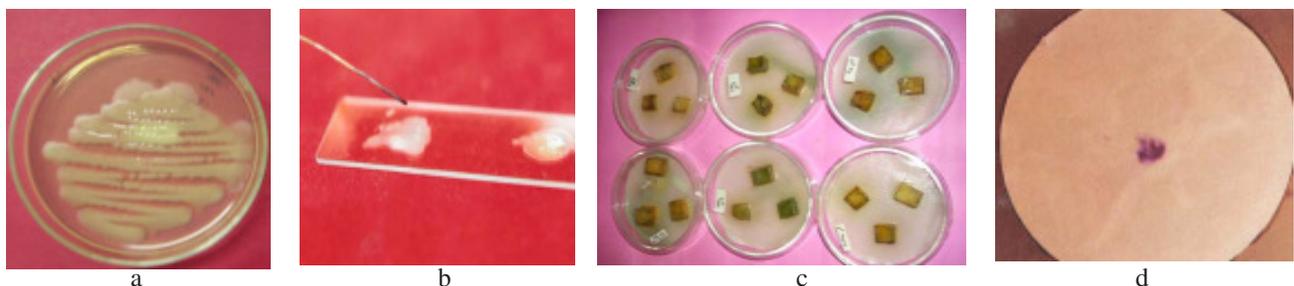


Fig 2 Representative results of physiological tests on suspected isolates of *Clavibacter michiganensis* subsp. *michiganensis* obtained from various tomato production centers in Sumatra and Java; a, morphology of the bacterial colony suspected as *C. michiganensis* subsp. *michiganensis* on TSA medium 24 hours after plating; b, Positive results of Gram test using KOH solution on isolate SLK 11 from Danau Kembar, Solok, West Sumatera; c, ectinase test on isolate AGM-3 from Baso, Agam, West Sumatera; and d, oxidation test on isolate RJL-74 from Talang Rimbo, Kepahiang, Bengkulu.

subsp. *michiganensis* infection in tomato plants indicated that the incidence of suspected *C. michiganensis* subsp. *michiganensis* infection ranged from 1-20% (Table 1). The highest disease incidence (up to 20%) was observed at Pejawan, Banjarnegara, Central Java, and at Pujon, Malang, East Java (Table 1). Disease incidence ranging from 10-12% was observed at Ngantung, Malang, East Java; Wanayasa, Banjarnegara, Central Java; Baso and Banuhampu, Agam, West Sumatra; and Peceran, Berastagi/Karo, North Sumatra. An incidence of up to 5-7% was observed at Cipanas and Pacet, Cianjur, West Java. Other locations surveyed indicated that the incidence of suspected *C. michiganensis* subsp. *michiganensis* infection was less than 5% (Table 1).

In the various locations surveyed, the tomato cv. Marta was the most common of the tomato cultivars that exhibited symptoms of *C. michiganensis* subsp. *michiganensis* infection. This cultivar is recommended for cultivation at high altitude. Other tomato cultivars exhibiting symptoms of *C. michiganensis* subsp. *michiganensis* infection in the field were the tomato cvs. Permata, Montera, and Cosmonot. These four tomato cultivars were the most commonly grown cultivars in North Sumatra, West Sumatra, Bengkulu, East Java, and West Java.

Isolation of *C. michiganensis* subsp. *michiganensis* from Tomato Samples. Bacterial isolates grown on NA medium exhibited many different colony morphologies. Of the colonies evaluated, only 24 indicated similar morphologies as the reference *C. michiganensis* subsp. *michiganensis* isolate 542 and these 24 isolates were selected for further experiments.

To obtain a pure single colony, the 24 isolates were spread onto YDC medium and incubated for 24-48 h at room temperature. The expected *C. michiganensis* subsp. *michiganensis* colonies on YDC medium should be slow

growing, mucous, and yellow to pale-orange in color (Anwar et al. 2005). Results of the single colony purification showed that six isolates were not *C. michiganensis* subsp. *michiganensis* and only 18 isolates exhibited a yellow color and were mucous and wet colonies (Fig 2a). The other six bacterial isolates were fast growing and neither showed mucous nor yellow to pale colonies on YDC medium.

Identification of Bacterial Isolates. Various physiological tests were conducted to confirm the identity of the isolates as *C. michiganensis* subsp. *michiganensis*. Results of evaluation indicated 18 selected isolates were gram positive (Table 2). Results also indicated those 18 isolated all showed similar results of physiological tests as the *C. michiganensis* subsp. *michiganensis* isolate 542 (Table 2), indicating they were *C. michiganensis* subsp. *michiganensis*.

The control *C. michiganensis* subsp. *michiganensis* isolate 542 clearly induce positive HR response in leaves of both *N. tabacum* and *M. jalapa* (Table 2). Results of the HR tests showed that 17 out of 18 isolates also induced the HR response in leaves of both species (Table 2). One isolate (KAR19), although physiologically exhibited similar characters to *C. michiganensis* subsp. *michiganensis* isolate 542, did not induce HR response on either *N. tabacum* or *M. jalapa*.

Results of the pathogenicity tests further confirmed the identity of the isolates as *C. michiganensis* subsp. *michiganensis*. Inoculation of 11 out of 18 isolates onto tomato seedlings resulted in symptoms similar to that of *C. michiganensis* subsp. *michiganensis* isolate 542 (Table 2) and these 11 isolates were positively identified as *C. michiganensis* subsp. *michiganensis*. Although showing inconsistent results, pathogenicity tests of the other six isolates also positively identified them as *C. michiganensis* subsp. *michiganensis*. After the inoculation

Table 2 Characteristics of bacterial isolates suspected as *Clavibacter michiganensis* subsp. *michiganensis* from various tomato production centers in Sumatera and Java

Isolate code number	Origin of suspected bacterial isolates	Characteristics of isolate									
		A	B	C	D	E	F	G	H	I	
KAR-15	Peceran, Berastagi/Karo	+	+	+	+	+	-	-	+	+	
KAR-17	Peceran, Berastagi/Karo	+	+	+	+	*	-	-	+	+	
KAR-19	Peceran, Berastagi/Karo	+	+	+	-	*	-	-	+	+	
KAR-22	Logumba, Berastagi/Karo	+	+	+	+	+	-	-	+	+	
RJL-74	Talang Rimbo, Kepahiang	+	+	+	+	+	-	-	+	+	
AGM-1	IV Angkek, Agam	+	+	+	+	+	-	-	+	+	
AGM-3	Baso, Agam	+	+	+	+	+	-	-	+	+	
AGM-7	Banuhampu, Agam	+	+	+	+	+	-	-	+	+	
SLK-9	Danau Kembar, Solok	+	+	+	+	+	-	-	+	+	
SLK-11	Danau Kembar, Solok	+	+	+	+	+	-	-	+	+	
SLK-13	Lembah Gumanti, Solok	+	+	+	+	*	-	-	+	+	
TND-5	Tanjung Baru, T. Datar	+	+	+	+	*	-	-	+	+	
CJR-45	Cipanas, Cianjur	+	+	+	+	*	-	-	+	+	
CJR-53	Pacet, Cianjur	+	+	+	+	*	-	-	+	+	
BJN-28	Wanayasa, Banjarnegara	+	+	+	+	-	-	-	+	+	
MLG-65	Pujon, Malang	+	+	+	+	+	-	-	+	+	
MLG-66	Kepung, Kediri	+	+	+	+	+	-	-	+	+	
KDR-68	Matus, Kediri	+	+	+	+	+	-	-	+	+	
<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i> 542	PRI, Wageningen-Holland	+	+	+	+	+	-	-	+	+	

A, Gram reaction test; B, pectinase test; C, oxidation test; D, hypersensitive response (HR) test; E, pathogenicity test on seedlings of tomato cv. Marta; F, fluorescent pigment test on King's B medium; G, nitrate reduction test; H, growth on TTC medium; I, tolerance against 6% of NaCl; *Showed inconsistent HR test results among tomato seedlings tested.

of tomato seedlings with these six suspected isolates, some seedlings showed symptoms typical of *C. michiganensis* subsp. *michiganensis* infection while the others did not. Moreover, the inoculated seedlings also exhibited various severities of symptoms.

Although isolate KAR19 did not induce the HR response, tomato seedlings inoculated with this isolate exhibited typical symptoms of *C. michiganensis* subsp. *michiganensis* infection in at least one tomato seedling. Such results indicate that isolate KAR19 was also *C. michiganensis* subsp. *michiganensis*. Inoculation of the BBN28 isolate onto tomato seedlings resulted in negative symptoms for *C. michiganensis* subsp. *michiganensis* infection (Table 2). However, physiological characters of isolate BBN28 were similar to that of *C. michiganensis* subsp. *michiganensis* isolate 542 and it did induce the HR response in *N. tabacum* and *M. jalapa* (Table 2). Further pathogenicity tests need to be conducted to confirm the identity of isolate BBN28. This remains to be done.

Six bacterial isolates identified as non-*C. michiganensis* subsp. *michiganensis* based on their colony morphologies also exhibited different physiological characteristics compare to that of *C. michiganensis* subsp. *michiganensis* isolate 542. The six non-*C. michiganensis* subsp. *michiganensis* bacterial isolates neither induced the HR response in leaves of *N. tabacum* and *M. jalapa* nor the typical symptoms of *C. michiganensis* subsp. *michiganensis* infection in tomato seedling (Data not presented). Such results confirmed the identity of these isolates as non-*C. michiganensis* subsp. *michiganensis*.

DISCUSSIONS

The introduction of a new disease into certain regions or countries such as Indonesia is one of the many consequences of trading and exchanging seeds between countries (Chang *et al.* 1989; Chang *et al.* 1991). Free seed importation to Indonesia and germplasm exchange for breeding programs in the year 2000 might possibly have introduced certain pathogens previously absent in Indonesia, such as *C. michiganensis* subsp. *michiganensis*. The results of this experiment further indicated that *C. michiganensis* subsp. *michiganensis*, the causal agent of bacterial canker in tomato, have existed in Indonesia, and spread in a number of tomato production centers in Sumatra and Java.

Positive results of *C. michiganensis* subsp. *michiganensis* identification from suspected tomato samples should be seen as a warning sign for all tomato production stakeholders in Indonesia, especially those responsible in regulating the seed trade, importation and plant quarantine.

In a number of locations, *C. michiganensis* subsp. *michiganensis* infection occurred only in one or two plants, indicating that the spreading of this pathogen was still limited. However, if not managed correctly, such conditions might develop into wide spread infections among tomato plantations and result in the outbreak of bacterial canker that would cause substantial losses to tomato growers. Therefore, tomato growers in the surveyed regions should

be aware of this newly introduced tomato disease in order to prevent possible outbreaks of bacterial canker. Outbreaks of bacterial canker due to *C. michiganensis* subsp. *michiganensis* infection has been reported in various countries (Hausbeck *et al.* 2000; Sahin *et al.* 2002).

Observed symptoms of *C. michiganensis* subsp. *michiganensis* infection in the field include wilting leaves and necrosis at the leaf perimeters (Basim *et al.* 2004), fruit showing bird's eye spot symptoms (Medina-Mora 2001), as well as split stem and stem canker (Burokienė *et al.* 2005). Wilting-leaf-symptoms in the tomato plant occur because *C. michiganensis* subsp. *michiganensis* infection develops unilaterally from the lower position to an upper one on the stem and finally damages all of the leaves. In these experiments, bird's eye spot symptoms were observed at Danau Kembar, Solok, West Sumatera; split stem secretions and pith discoloration were observed at Lembah Gumanti, Solok, West Sumatera; wilting leaves and necrosis at the leaf perimeters at Matus, Kediri, East Java; blackened stem, wilting leaves and necrosis at the leaf perimeters were observed at Wanayasa, Banjarnegara, Central Java; and stem necrosis (tissue discoloration) was observed at Cipanas, Cianjur, West Java.

The inconsistencies between field survey and laboratory observations could not rule out the fact that *C. michiganensis* subsp. *michiganensis* has positively been identified in a number of surveyed locations. Symptoms of *C. michiganensis* subsp. *michiganensis* infection in the field were often similar to that of *Xanthomonas* spp. (Gram negative bacterium) infection in tomato. In such cases, field surveys based on symptoms might result in higher disease incidences than that positively identified as *C. michiganensis* subsp. *michiganensis* infection based on laboratory studies. Any mistakenly identified symptoms in the field caused by *Xanthomonas* spp. could easily be eliminated in laboratory by the simple Gram reaction test, since *Xanthomonas* spp. is Gram negative while *C. michiganensis* subsp. *michiganensis* is Gram positive.

Furthermore, the HR test using leaves of *N. tabacum* and *M. jalapa* (Gitaitis 1990) could be used as quick indicators to verify the presence of *C. michiganensis* subsp. *michiganensis*. Using these HR tests, positive *C. michiganensis* subsp. *michiganensis* identification could be done only after 12 h of inoculation of the suspected bacterium into the leaves of *N. tabacum* and *M. jalapa*. On the other hand, pathogenicity testing exhibited varied results in this research. The expected positive identification of *C. michiganensis* subsp. *michiganensis* was inoculated tomato seedlings showing wilting symptoms for cotyledons, hypocotyls, death of the inoculated seedlings (Gitaitis *et al.* 1991; Anwar *et al.* 2005). However, in some cases in these experiments, such clear cut results were not observed and the inoculated tomato seedlings did not exhibit clear symptoms of *C. michiganensis* subsp. *michiganensis* infection.

The inconsistencies in the pathogenicity test might be because (i) environment conditions might not be optimum for disease development after *C. michiganensis* subsp. *michiganensis* inoculation, (ii) tomato cv. Marta might not be the best indicator for the pathogenicity test, and (iii) the tested isolates might exhibit different degrees of

virulence against tomato cv. Marta. In this research, the pathogenicity test was conducted in a glasshouse. The temperature in the glasshouse sometimes reaches 30°C. Such temperature might not be suitable for disease development after *C. michiganensis* subsp. *michiganensis* infection.

The most common tomato cultivar used for the pathogenicity test against *C. michiganensis* subsp. *michiganensis* is the highly susceptible tomato cv. Money Maker. This tomato cultivar, however, is not available in Indonesia. Therefore, the commonly grown tomato cultivar Marta was used as the indicator in this research. The response of tomato cv. Marta against *C. michiganensis* subsp. *michiganensis* infection has not been reported elsewhere. A number of suspected *C. michiganensis* subsp. *michiganensis* isolates were tested and the results of pathogenicity test were inconsistent. However, results of this research indicated that the tomato cultivar Ratna was susceptible to infection of most of the *C. michiganensis* subsp. *michiganensis* isolates identified. Differences in virulence among *C. michiganensis* subsp. *michiganensis* isolates might result in inconsistencies in pathogenicity testing (Berry *et al.* 1989). It seems possible that the identified *C. michiganensis* subsp. *michiganensis* isolates from the various tomato production centers in Sumatra and Java consisted of many different isolates with different degrees of virulence against the tomato cv. Marta. To test this hypothesis, however, further analysis of the identified *C. michiganensis* subsp. *michiganensis* isolates using molecular markers is required. Further identification of isolated *C. michiganensis* subsp. *michiganensis* isolates will be conducted and the results will be presented in later reports.

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