PHENOTYPIC CHARACTERIZATION OF CLAVIBACTER MICHIGANENSIS SUBSP. MICHIGANENSIS ISOLATES FROM LITHUANIA

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Abstract

14 isolates of Clavibacter michiganensis subsp. michiganensis obtained in 2003–2004 from tomato and eggplant grown in different fields and greenhouses in Lithuania were studied. Our studies revealed that the morphological, biochemical and physiological characteristics of the strains examined did not differ. All of them were similar to those described in “Bergey’s manual...” (1994), except for the ability to induce slight reaction in utilization of melezitose to produce acid. On the basis of pathogenicity tests on tomato seedlings, all isolates were divided into three groups: high, medium and low virulence. It was found that height of inoculated tomato plants influenced development of bacterial canker symptoms. Inoculum concentration also influenced the occurrence of disease symptoms. Inoculation of tomato plants through wounded roots indicated that at least 33 days were necessary for the first symptoms of bacterial canker to emerge. However, regardless of inoculation method (root or stem) disease development depended on the virulence of the studied isolates.

Key words: Clavibacter michiganensis subsp. michiganensis, tomato, pathogenicity, conventional determination

Introduction

Clavibacter michiganensis subsp. michiganensis (Smith) Davis et al. (Cmm), the causal agent of bacterial canker of tomato, is a quarantine organism occurring in...
some areas within European Union ("Clavibacter michiganensis..." 2005). The pathogen is spread to disease-free areas via infected seeds and transplants (Chang et al. 1989, Gitaitis et al. 1991). The severity of disease is very high, the presence of less than 1% infected seeds can cause 60–70% crop loss, i.e. epidemics in the fields and greenhouses can be induced by few plants (Bryan 1930, Chang et al. 1991, Strider 1969).

During last three–four years, bacterial canker has become an economically important disease of tomatoes grown in Lithuania’s greenhouses. In some cases the incidence of the disease reached 10% (Vasinauskienė 2002) to 80% of cultivated tomato plants (Burokienė unpublished).

The first foci of tomato bacterial canker in Lithuania were found over 70 years ago (Vilkaitis 1933, Brundza 1935). At the time its diagnosis was based only on presence of characteristic symptoms of wilting plants.

The objective of the present study was to determine morphological, physiological, biochemical and pathogenic properties of Cmm-like isolates originating from symptomatic tomato and eggplants grown in various regions of Lithuania during 2003–2004.

Materials and methods

Bacteria

12 Cmm-like isolates were obtained from diseased tomato plants (Lycopersicon esculentum L.). Five of them: Cmm 5, Cmm 6, Cmm 10, Cmm 11 and Cmm 13 were derived from plants grown under field conditions, and seven: Cmm 3, Cmm 4, Cmm 7, Cmm 8, Cmm 9, Cmm 12 and Cmm 14 – from plants grown in the greenhouse. Two other isolates marked as Cmm 1 and Cmm 2, were obtained from eggplants (Solanum melongena L.) also grown in the greenhouse. For comparison, two reference strains of Clavibacter michiganensis subsp. michiganensis (Cm 78-S and Cm 8) kindly supplied by Dr. E. Griesbach from Federal Centre for Breeding Research on Cultivated Plants, Institute of Resistance Research and Pathogen Diagnostics, Aschersleben, Germany, were included in this study.

Bacteria were cultivated on the following media: nutrient dextrose agar (NDA) (nutrient agar 2.3%, D-glucose 1.0% in distilled water), self made potato dextrose agar (PDA) (peeled washed potato 500 g/l of water, D-glucose 1.0%, Oxoid agar (No 3) 1.5%) and yeast glucose mineral agar (YGMA) (yeast extract 0.2%, glucose 0.25%, K2HPO4 0.025%, KH2PO4 0.025%, MgSO4·7H2O 0.01%, MnSO4 0.0015%, NaCl 0.005%, FeSO4·7H2O 0.0005%, Oxoid agar (No 3) 1.5% in distilled water). Depending on the test, bacteria were incubated for two–five days at 25°C. During the period of study, bacteria were maintained on slants with NDA medium at 4°C.
Screening of bacteria pathogenicity

Hypersensitivity reaction (HR)

All Cmm-like isolates were tested for ability to induce HR on tobacco plants according to the method of Klement (1963). Water bacterial suspension of each isolate was infiltrated by medical syringe into the mesophyll of a fully expanded tobacco leaf cv. ‘Samsun’. HR was determined after 24 h.

Pathogenicity on tomato seedlings

Inoculum of each isolate and strain was prepared from two-day-old bacterial cultures grown on NDA slants. The bacteria were suspended in phosphate-buffered saline (PBS) (Na₂HPO₄ 0.27%, NaH₂PO₄ 0.04%, NaCl 0.8%, pH 7.2 in distilled water). Inoculum concentration was adjusted to 10⁸ cfu/ml using spectrophotometer SEMCO S91E (EMCO, Poland) serial dilution plate method on NDA medium. The test was performed on two-week-old tomato seedlings cv. ‘Monika’ at stage of one to two true leaves. Five plants were inoculated with each isolate by stabbing the stem above the first leaf with a needle previously dipped in inoculum (Foster and Echandi 1973). Inoculated plants were covered with polyethylene bags for 48 h and kept in a chamber at relative humidity of 85% with a 16/8 h day/night photoperiod and temperature of 25/18°C. The occurrence of bacterial canker symptoms was recorded in each of three weeks, but no evaluation of disease severity was made. The isolation of bacteria on NDA medium from one of each symptomatic plant, representing each isolate, was performed.

Determination of physiological and biochemical characters of bacteria

The determination of physiological and biochemical features of Cmm-like isolates and reference strains were performed according to “Bergey’s manual...” (1994) and Bradbury (1986).

Gram reaction

Staining of bacteria with a crystal violet, then with safranin was performed according to method described in “Laboratory guide...” (2001). Additionally, a Gram reaction was conducted by mixing bacteria with a drop in 3% solution of KOH (Suslow et al. 1982).

Utilization of carbon compounds

Oxidative/Fermentative Test (O/F). A basal medium (Hugh and Leifson 1953) (peptone 0.2%, NaCl 0.5%, K₂HPO₄ 0.03%, Oxoid agar (No. 3) 0.3%, bromthymol blue 0.003% in distilled water, pH 7.1) containing 1% glucose was incubated for 14 days. The production of a yellow colour (indicating acid production) in the unsealed tube, but not the sealed tube, indicates oxidative metabolism of glucose.

Levan production from sucrose. Bacteria were streaked onto Nutrient Agar (NA) medium supplemented with 5% sucrose. High-domed, shining and mucoid colonies after six days of incubation indicated a positive reaction (Lelliott and Stead 1987).
Acid production from carbohydrates. The yellow colouring of synthetic base containing 1% of sugar (cellobiose, inulin, manrose, melezitose, mannitol or sorbitol) after 14–28 days of bacteria incubation on it indicated positive result (Dye 1968).

Utilization of organic acids. The yellow colouring of synthetic medium containing 1% of acetate, citrate, propionate or succinate, after 14–20 days of bacteria incubation on it indicated a positive result (Ayers et al. 1919).

Degradation of macromolecules

Gelatin hydrolysis. Bacteria were grown in test tubes containing NB (Nutrient Broth) medium with 12% of gelatin for up to 28 days at 20°C (Lelliott and Stead 1987). The result was negative if liquefaction of medium did not occur.

Soluble starch hydrolysis. Bacteria grown on NA medium containing 0.2% soluble starch (w/v) (“Methods in phytobacteriology” 1990). The absence of clear zone around the colonies was an indication of absence of hydrolysis after 10 days.

Esculin hydrolysis. Bacteria were incubated for three days at 25°C on agar medium containing esculin (Lelliott and Stead 1987). The brown colouring of medium indicated positive reaction.

Other tests

Presence of catalase. Two-day-old bacteria grown on NA medium were transferred on a glass slide and mixed with a drop of freshly prepared 3% H₂O₂. Formation of bubbles indicated a positive reaction (“Laboratory guide…” 2001).

Presence of oxidase. A loopful of bacteria grown on NDA medium was transferred on a filter impregnated with freshly made 1% aqueous solution of tetramethyl-p-phenylenediamine dihydrochloride. If no purple colour developed after 60 s, the isolate was rated oxidase-negative (Kovacs 1956).

Evaluation of bacteria virulence

The study was carried out by inoculation of tomato plants cv. ‘Monika’ grown for five weeks in plastic pots (8 cm in diameter) containing a mixture of 4/5 soil substrate (AGROHUM, Poland) and 1/5 sterilized sand. The inoculum was standardized using the method described above. Tomato plants (five per each isolate: one at height of 20–25 cm, three at 25–30 and one at 30–35 cm) were inoculated by stabbing the stem above the second and the third leaf with a needle previously dipped in 10⁸ cfu/ml bacterial suspension of each isolate.

Inoculated plants were covered with polyethylene bags for 48 h. They were kept in a chamber at relative humidity of 85% with a 16/8 h day/night photoperiod and temperature of 25/18°C.

Inoculated plants were observed daily until 23 days after inoculation. One sided wilting was used as the diagnostic feature. Evaluation of disease appearance and development was determined using the scale of Foster and Echandi (1973) with some modifications: 0 – no symptoms, 1 – up to 1/3 of the leaves wilted, 2 – up to 2/3 of the leaves wilted, 3 – more than 2/3 of wilted leaves but the terminal leaves
on the main shoot not wilted, 4 – terminal leaves of the main shoot and most leaves wilted or dead (Phot. 1). The intensity of bacterial canker was evaluated on the basis of disease severity index (DSI).

All data were subjected to an analysis of variance and separation of means using Duncan’s t-test. A linear regression analysis ($y = a + bx$) was applied for determination of the relationship between severity of disease and height of tomato plants.

**Infection threshold**

The study with the Cmm 1 isolate and the Cm 78-S strain was carried out by inoculation of tomato plants cv. ‘Monika’ grown for five weeks in plastic pots (8 cm in diameter) containing a mixture of 4/5 soil substrate (AGROHUM, Poland) and 1/5 sterilized sand. The inoculum was standardized using the method described above; a series of 10-fold dilutions in PBS buffer ranging from $10^2$ to $10^8$ cfu/ml
were prepared. Inoculated plants (five per each isolate/strain) were covered with polyethylene bags for 48 h. They were kept in a chamber at relative humidity of 85% with a 16/8 h day/night photoperiod and temperatures of 25/18°C. Inoculated plants were observed daily for appearance of typical symptoms for 24 days after inoculation.

Plants infection through wounded roots

Root inoculation of five-week-old tomato plants cv. ‘Monika’ was performed by introduction of 1 ml of $10^8$ cfu/ml inoculum into a hole made by a sterile scalpel in the soil substrate (see above) 4 cm along one side of the plant stem grown in plastic pot to a 4 cm depth (soil drench). The plants were incubated in a moist greenhouse chamber (relative humidity 80–85%) with 16/8 h day/night photoperiod and temperature 25/18°C. For the first 48 h the plants were covered with polyethylene bags. All Cmm-like isolates and reference strains were used in this study. For each of treatment, roots of five plants were inoculated. Control plants were treated in the same way with sterile PBS buffer.

Results

Pathogenicity

All tested isolates caused necrosis of tobacco leaf tissue between veins after 24 h from its infiltration, which indicates typical HR reaction (Phot. 2 a). All isolates also produced typical symptoms of tomato bacterial canker – one sided wilt of tomato seedlings on 14th day after stem inoculation (Phot. 2 b). Also, reisolations of Cmm-like bacteria from tomato plants were positive in all cases.

Phot. 2. a. All Clavibacter michiganensis subsp. michiganensis like isolates induced hypersensitivity reaction on tobacco. b. All Clavibacter michiganensis subsp. michiganensis like isolates produced typical symptoms of bacterial canker on tomato seedlings (one side wilting of leaves) after inoculation with water suspension of $10^8$ cfu/ml – right; control – left (photo by D. Burokiené)
Phenotypic characteristics of Cmm-like isolates

After four days of incubation on YGMA medium the colonies of tested isolates were rated as convex, mucoid, pale-yellow to yellow. They were slow-growing – approximately 2–3 mm in diameter.

Morphological, biochemical and physiological features of studied isolates and reference strains Cm 8 and Cm 78-S were similar (Table 1). All isolates were gram-positive coryneform rods with oxidative metabolism, not producing oxidase and levan but positive for catalase. They also hydrolized esculin and very weakly

<table>
<thead>
<tr>
<th>Biochemical test</th>
<th>“Bergey’s manual...”1994</th>
<th>Reference strains Cm 8 and Cm 78-S</th>
<th>Cmm 1 – Cmm 14 isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram reaction – stain</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Gram reaction – 3% KOH</td>
<td>nd</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Colonies with:</td>
<td></td>
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</tr>
<tr>
<td>yellow or orange pigment</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>blue and grey pigment</td>
<td>D</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Levan</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>esculin</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>soluble starch</td>
<td>D</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>gelatine</td>
<td>+w</td>
<td>+w</td>
<td>+w</td>
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<tr>
<td>Acid is produced from:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cellobiose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>inulin</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>mannitol</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>mannose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>melezitose</td>
<td>–</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>sorbitol</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Utilization of organic acid:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>acetate</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>citrate</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>propionate</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>succinate</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>O/F test</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

+ – 90% or more of strains were positive, – – 90% or more of strains were negative, D – 11–89% of strains were positive, +w – weak reaction, nd – no data.
gelatin, produced acid from cellobiose, mannose and very weakly from melitzitose as well as utilized acetate, citrate and succinate.

**Virulence**

Disease severity on inoculated tomato seedlings depended on isolate or strain tested. Ratings performed 17 or 23 days after inoculation allowed distinguishing two or three groups, respectively (Fig. 1). The low disease severity was caused by reference strains Cm 8 and Cm 78-S, as well as by isolates originating from to-

![Graph showing disease degree vs. plant height](image-url)

**Fig. 1.** Evaluation of disease degree on tomato seedlings cv. ‘Monika’ 17 and 23 days after inoculation with *Clavibacter michiganensis* subsp. *michiganensis*; 1 – strain Cm 8, 2 – strain Cm 78-S, 3–16 – isolates Cmm 1 – Cmm 14

![Graph showing relationship of disease degree to plant height](image-url)

**Fig. 2.** Relationship of disease degree to plant height by linear regression
mato plants (Cmm 6, Cmm 10 and Cmm 14) and from eggplant (Cmm 2). On the contrary, the second isolate derived from eggplant (Cmm 1) gave the highest mean of disease severity as compared to other tested isolates. Moreover, it was found that the height of the plant used in this study influenced the development of symptoms – smaller plants showed wilting symptoms earlier and died faster than taller plants (Fig. 2).

**Infection threshold**

The inoculum concentration influenced occurrence of disease symptoms. All five plants inoculated with the highest concentrations of two strains used wilted earlier and showed almost the same reaction both at $10^7$ and $10^8$ cfu/ml in comparison to plants inoculated with lower concentrations. Furthermore, analysis indicated, that plants inoculated with $10^2$ cfu/ml and $10^3$ cfu/ml concentrations needed more time to develop symptoms than those inoculated with concentrations $10^4$–$10^8$ cfu/ml. Only one or two plants showed wilting symptoms during the course of experiment, after inoculation with $10^2$ or $10^3$ cfu/ml, respectively. The time needed to develop symptoms also depended on the isolate virulence; more virulent Cmm 1 showed symptoms earlier than reference strain Cm 78-S (Table 2).

**Table 2**

The influence of inoculum concentration of *Clavibacter michiganensis* subsp. *michiganensis* on the presence of bacterial canker symptoms on tomato plants (number of plants with symptoms)

<table>
<thead>
<tr>
<th>Strain, isolate</th>
<th>Inoculum concentration</th>
<th>Days after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td><strong>Cm 78-S</strong></td>
<td>$10^8$</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$10^7$</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$10^6$</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$10^5$</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$10^4$</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$10^3$</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$10^2$</td>
<td>0</td>
</tr>
<tr>
<td><strong>Cmm 1</strong></td>
<td>$10^8$</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$10^7$</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$10^6$</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$10^5$</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$10^4$</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$10^3$</td>
<td>0</td>
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<tr>
<td></td>
<td>$10^2$</td>
<td>0</td>
</tr>
</tbody>
</table>
Roots infection

Inoculation of tomato plants with Cmm through wounded roots indicated, that at least 33 days were necessary to emerge the first symptoms of bacterial canker. Isolate Cmm 1 induced disease symptoms on all five inoculated plants during the period of 39 days after inoculation while isolate Cmm 7 on four plants in the same period. No wilting symptoms on any plant were observed after inoculation with Cmm 13 (Table 3).

Table 3
The effect of tomato plants root inoculation on development of bacterial canker (number of plants with symptoms)

<table>
<thead>
<tr>
<th>Strain, isolate</th>
<th>Days after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>33</td>
</tr>
<tr>
<td>Cm 8</td>
<td>0</td>
</tr>
<tr>
<td>Cm 78-S</td>
<td>0</td>
</tr>
<tr>
<td>Cmm 1</td>
<td>1</td>
</tr>
<tr>
<td>Cmm 2</td>
<td>0</td>
</tr>
<tr>
<td>Cmm 3</td>
<td>1</td>
</tr>
<tr>
<td>Cmm 4</td>
<td>0</td>
</tr>
<tr>
<td>Cmm 5</td>
<td>0</td>
</tr>
<tr>
<td>Cmm 6</td>
<td>0</td>
</tr>
<tr>
<td>Cmm 7</td>
<td>3</td>
</tr>
<tr>
<td>Cmm 8</td>
<td>0</td>
</tr>
<tr>
<td>Cmm 9</td>
<td>1</td>
</tr>
<tr>
<td>Cmm 10</td>
<td>0</td>
</tr>
<tr>
<td>Cmm 11</td>
<td>1</td>
</tr>
<tr>
<td>Cmm 12</td>
<td>1</td>
</tr>
<tr>
<td>Cmm 13</td>
<td>0</td>
</tr>
<tr>
<td>Cmm 14</td>
<td>1</td>
</tr>
</tbody>
</table>

Discussion

Our study showed that all isolates studied, originating from diseased tomato plants and eggplants grown in the fields and greenhouses in Lithuania, belonged to Clavibacter michiganensis subsp. michiganensis. They were very homogeneous in morphological, physiological and biochemical characteristics and did not differ from the reference strains obtained from Dr. E. Griesbach (Aschersleben, Germany) and characteristics published by Bradbury (1986) and Holt et al. (eds – “Bergey’s manual…” 1994). However, there was a difference in the ability to induce slight reaction in utilization of melezitose, which indicates production of acid. This finding is simi-
lar to that of Davis et al. (1984). Also, 10% out of about 100 strains of Cmm studied by Kamasa (2004) showed this property. However, Behrendt et al. (2002) studying a representative selection of coryneform bacteria found that *Clavibacter michiganensis* subsp. *michiganensis* did not utilize melezitose. All studied Lithuanian and reference strains hydrolyzed very weekly gelatine after four weeks of incubation.

The pathogenicity and virulence of Cmm isolates presented in this work demonstrated that plant origin of bacteria did not influence their virulence when tested on tomato seedlings. It should be pointed out that isolate Cmm 1, originated from eggplant, caused the highest disease severity as compared to other tested isolates. It induced symptoms earliest; first symptoms on tomato appeared already eight days after inoculation. On the contrary, Bogo and Takatsu (1997), working with isolates from tomato and pepper, showed a high degree of correlation between virulence and host origin which demonstrated a clear host preference.

Virulence studies indicated that smaller plants showed wilting symptoms earlier and died faster, i.e. plant height influenced symptoms development. However, it is well known, that height of tomato seedlings depends on plant age – older plants are higher than younger. Chang et al. (1992) and Foster and Echandi (1973) noted that incubation period was longer and severity of disease decreased as plant age increased. Our studies on virulence showed, that with plants at the same age, but of different height, symptoms development was inversely proportional to plant height, i.e. when plant height increased – disease severity decreased.

Differences regarding disease development were related to virulence of tested isolates. Several studies have been published describing the mechanisms involved in inducing bacterial wilt (Jahr et al. 1999, Gartemann et al. 2003, Meletzus et al. 1993). One of the factors causing plant wilting is production of exopolysaccharides (Billing 1987, Denny 1995, Kiraly et al. 1997, Van Alfen et al. 1987, van den Bulk et al. 1989), as well as, some extracellular enzymes such as endocellulase (Meletzus et al. 1993), pectinmethylesterase (Strider 1969), xylanase (Beimen et al. 1992), hydralase (Benhamou 1991). For the development of disease symptoms on infected tomato plants the pathogenicity locus *pat-1* (Burger et al. 2005, Dreier et al. 1997) and *celA* gene encoding endoglucanase (Jahr et al. 1999, 2000, Metzler et al. 1997) are required.

The investigations of bacteria virulence indicated that inoculum concentration affected symptom development. Generally, leaves on inoculated seedlings wilted faster at higher applied concentrations (10^7 and 10^8 cfu/ml), and longer time was needed for wilt development at lower concentrations (10^2–10^3 cfu/ml). It should be pointed out that even at the lowest concentrations of bacteria in inoculum, plants were infected and wilting occurred. The results of this study confirm earlier reports (Boelema 1976, 1977, Berry et al. 1989, Chang et al. 1992, Foster and Echandi 1973, Thyr 1972). In our experiments, with two strains of different virulence, but the same inoculum concentration (10^2 cfu/ml), the symptoms appeared on one out of five plants inoculated with a strain. However, the symptoms were visible two days earlier in the case of more virulent strain Cmm 1. On the contrary, Chang et al. (1992) published, that symptoms could develop when inoculum concentration was 80 cfu/ml, but incubation period could be prolonged if plants were
inoculated with lower concentrations of bacteria. Thyr (1968) described that tomato seedlings could become infected by Cmm with as few as five cells per plant when they were introduced directly into xylem tissue.

Our study showed that disease induction and development depended on the method of inoculation – plants inoculated through roots showed symptoms much later than plants inoculated directly to the stem. First symptoms were recorded just before 33 days after inoculation of wounded roots. Huang and Tu (1999, 2001) informed that the first symptoms of the disease appeared 39–49 days after inoculation of roots, but they used another root inoculation method: roots were clipped 5 cm from crown and dipped in a suspension of $3 \times 10^8$ cfu/ml of Cmm for 15 min. The authors also showed the importance of nutrient solution pH applied to the soil after inoculation. Conditions affecting Cmm survival and spread are also described by other authors (Basu 1970, Chang et al. 1990, Chitarra et al. 2000, Fatmi and Schaad 2002, Grogan and Kendrick 1953, Strider 1967). The root inoculation method failed to produce uniform symptom when bacterial suspension was poured over the uninjured or clipped roots of seedlings planted in the soil (Strider 1970). However, it should be stressed that soil contamination and disease occurrence on plants due to root infection remains very important, because Cmm can survive in soil and be transmitted through cultural practices (Chang et al. 1991, Fatmi and Schaad 2002, Strider 1967).

Streszczenie

CHARAKTERYSTYKA FENOTYPOWA IZOLATÓW CLAVIBACTER MICHIGANENSIS SUBSP. MICHIGANENSIS POCHODZĄCYCH Z LITWY

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