
Bacterial diseases of greenhouse-grown tomatoes

M. Vasinauskienė

*Institute of Botany,
Žaliųjų ežerų 49,
LT-2021 Vilnius, Lithuania*

Over the recent years bacterial diseases cause great crop losses to tomatoes grown in greenhouses of the country. The distribution of most harmful diseases such as tomato pith necrosis, bacterial cancer, and bacterial speck was determined. Pathogenic bacteria were isolated from injured plants and according to their morphological, physiological, and pathogenic characteristics identified as *Pseudomonas corrugata*, *Clavibacter michiganensis* subsp. *michiganensis*, and *Pseudomonas syringae* pv. *tomato*. The most severe damage for greenhouse-grown tomatoes in the country was caused by the vascular pathogens *Pseudomonas corrugata* and *Clavibacter michiganensis* subsp. *michiganensis*.

Key words: *Pseudomonas corrugata*, *Clavibacter michiganensis* subsp. *michiganensis*, *Pseudomonas syringae* pv. *tomato*, bacterial diseases, tomato, disease incidence

INTRODUCTION

Greenhouse-grown tomatoes are of great importance as the main greenhouse crop in Lithuania. Unfortunately, their crop very often suffers from diseases. Observations of recent years revealed the harmfulness of bacterial diseases in the greenhouses of the country. Bacterial diseases could be often confused with other diseases, and control measures such as fungicides or other pest-control chemicals usually used against fungal diseases have no effect on bacteria. Therefore, identification of the pathogen is required for determining the disease control measures.

The objective of this research was to identify most harmful bacterial diseases and their causal agents of greenhouse-grown tomatoes and to estimate their damage to tomato crop.

MATERIALS AND METHODS

Isolation and identification of the pathogens. Sampling was carried out in major greenhouses of the country. Pathogens responsible for the disease symptoms were isolated from the damaged parts of plants. Homogenized samples were streaked on potato agar (PA) and nutrient dextrose agar (NDA) media to isolate the bacteria [1]. Petri dishes were incubated at 27 °C for five days. Bacterial colonies were maintained on PA slants.

The identification of the bacterial isolates and their reisolates was carried out according to the morphological and physiological characteristics. Levan production, oxidase reaction, potato soft rot, argini-

ne dihydrolase, tobacco hypersensitivity (LOPAT tests), Gram staining were evaluated [2–6]. The reference strains *Pseudomonas tomato* RO, *P. tomato* 034, and *Clavibacter michiganensis* subsp. *michiganensis* 78-S obtained from the Collection of Phytopathogenic Bacteria of the Institute for Epidemiology and Resistance (Ashersleben, Germany) were used as a positive control in the tests.

Pathogenicity tests. For determining the vascular pathogens, inoculations were carried out on four-week-old tomato seedlings at the stage of the second or third expanded leaf. Aqueous bacterial suspension of 10⁷ cfu/ml was used. Tomato seedlings were inoculated by injecting a drop of the bacterial suspension through the stem nodes into the pith. Inoculated plants were covered with polythene bags and incubated for 48 h before growing in a greenhouse [1, 7]. In six weeks, the external and internal symptoms were observed and bacteria reisolated. For the disease of leaf spot pathogens inoculum was applied onto the leaves.

RESULTS AND DISCUSSION

During the investigation three most harmful bacterial diseases – tomato pith necrosis, bacterial cancer, and bacterial speck – were determined and the associated pathogenic bacteria were isolated and identified.

Tomato pith necrosis. According to the study, tomato pith necrosis caused by *Pseudomonas corrugata* appeared to be most dangerous to tomato in greenhouses of the country. The first outbreak of the disease was registered in 1991. The crop losses

were estimated at 50%. After taking strict preventive measures the spread of disease was limited. However, disease occurrence in particular years ranged from 5 to 20%. The main reason for the spread was non-observance of phytosanitary regulations.

The disease was found on the 'Krasnaja zvezda', 'Dombella', 'Dručiai', 'Perkoz', and 'Svara' cultivars. The initial symptoms of the disease included chlorosis of young leaves. In the cases of high severity, chlorosis and wilting occurred on the top of plant and were accompanied by necrosis of the lower part of the stem. Infected stems had grey to dark brown lesions on their surface. Dark discoloration and cavities of the pith (the main symptoms of the disease) also occurred. A similar disease caused by *P. corrugata* was reported by C. M. Scarlett et al. [8].

Twenty bacterial strains isolated from samples of injured tomato plants were selected for identification. The tested isolates produced grey colonies with uneven edges and elevated centre on PA. They were Gram-negative aerobic bacteria showing positive results in the oxidase test. The majority of strains had positive tobacco hypersensitivity reaction. The bacteria did not rot potato slices. After inoculation of tomato seedlings, necrotic areas on the leaves and discoloration of stem pith appeared. No browning or pith necrosis occurred in control plants. The obtained data were similar to those in references [1, 8, 9]. Based on the analysis of disease symptoms and study of the morphological, physiological, and pathogenic characteristics of the isolated strains, the bacteria were identified as *P. corrugata* – the causal agent of tomato pith necrosis.

Bacterial cancer of tomato. In the recent years, bacterial cancer of tomato caused by *Clavibacter michiganensis* subsp. *michiganensis* has been recorded. Irregular wilting, shrivelling and browning of leaflets were found on injured plants and at first were frequently evident on only one side of leaves.

Bacteria were isolated from brown vascular tissues on NDA, a common medium for isolation and identification of bacteria. The isolated strains (14 isolates) were Gram-positive bacteria able to produce levan. On NDA they formed slow-growing mucoid, yellow to pale-orange colonies. After inoculation, wilt symptoms characteristic of bacterial cancer of tomato appeared on tomato seedlings.

Disease incidence approached 10%. The pathogen was found in the 'Raissa' cultivar. Bacterial cancer in Lithuania was recorded only in 1933–1934 so far [10, 11]. The disease is widespread in many countries [12]. Recent experiments carried out in France have shown yield losses of 20–30% [13]. *Clavibacter michiganensis* subsp. *michiganensis* is listed as an A₂ quarantine pathogen by the European and Mediterranean Plant Protection Organization. It is feared

that once the disease is induced, it is very difficult to eliminate the pathogen. Therefore, further special monitoring programs for eradication of this seed-born pathogen have to be started.

Bacterial speck of tomatoes. During the investigations bacterial strains were isolated from leaves and stems with typical symptoms of bacterial speck of tomatoes: small brown spots with yellow margins on tomato leaves and dark brown spots stretched along the stem. The pathogenicity of the six isolated strains was tested on host plants, and typical symptoms of the disease were revealed. The isolated strains were Gram-negative, aerobic, rod-shaped, motile bacteria. Colonies on PA were grey, circular with the elevated centre and uneven edges. Bacteria did not grow on potato slice, expressed inability to produce oxidase and arginine dihydrolase, and induced hypersensitivity reaction of tobacco leaves. Comparison of the characteristics of isolated strains with those described in literature [1, 14] and of the reference strains *P. tomato* RO and *P. tomato* 034 enabled to identify the bacterium as *Pseudomonas syringae* pv. *tomato* – the agent of bacterial speck of tomatoes. The disease is not widespread in Lithuania, only single injured plants of the cultivar 'Pirmutis' were found.

Therefore, on the basis of symptom analysis, pathogenic, morphological, and physiological characteristics, the isolated strains were identified as *Pseudomonas corrugata*, *Clavibacter michiganensis* subsp. *michiganensis*, and *Pseudomonas syringae* pv. *tomato*. The diseases they cause have been detected on the greenhouse-grown tomatoes and are distributed in the country. The most serious damage was caused by *P. corrugata* and *Clavibacter michiganensis* subsp. *michiganensis*. Considering the economic importance of tomato in Lithuanian vegetable growing, attention should be given to these vascular pathogens to avoid considerable losses like those that occurred in the recent years.

Only preventive control can reduce the risk of damage. It is important to avoid sprinkling irrigation, excess of nitrogenous fertilizers, and eliminate the infected plant debris. Use of healthy seeds is the first and most important condition for controlling these diseases, especially in case of seed-born bacteria such as *Clavibacter michiganensis* subsp. *michiganensis* or *Pseudomonas syringae* pv. *tomato*.

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M. Vasinauskienė

ŠILTNAMEIŲ AUGINAMŲ POMIDORŲ BAKTERINĖS LIGOS

S a n t r a u k a

Pastaraisiais metais Lietuvos šiltnamių ūkiuose išplito bakterinės pomidorų ligos. Iš pažeistų augalų buvo išskirtos fitopatogeninės bakterijos. Pagal jų morfologines, kai kurias fiziologines bei patogenines savybes buvo identifikuotos *Pseudomonas corrugata*, *Clavibacter michiganensis* subsp. *michiganensis* and *Pseudomonas syringae* pv. *tomato* bakterijos, sukeliančios pomidorų stiebo šerdies nekrozę, bakterinį vėžį ir bakterinę dėmėtligę. Nustatyta, kad ypač didelę žalą šiltnamiuose auginamiems pomidorams padaro vytulį sukeliančios bakterijos – *Pseudomonas corrugata* ir *Clavibacter michiganensis* subsp. *michiganensis*. Straipsnyje pateikiamos ir kai kurios svarbesnės apsaugos nuo šių ligų priemonės.