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RACES AND HOSTS OF *PSEUDOMONAS SYRINGAE* PV. *TOMATO* IN SERBIA

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Abstract — During the past few years, frequent appearance of bacterial speck of tomatoes was recorded in several tomato-growing regions in Serbia. A three-year survey of tomato fields in Serbia (2002-2004) resulted in the isolation of numerous bacterial strains, with 30 representative strains selected for further analyses. Based on the results of pathogenicity, biochemical, and physiological tests, all strains isolated from diseased tomato plants were identified as *P. syringae* pv. *tomato*. The identity of strains was confirmed by the polymerase chain reaction (PCR), since PCR products of expected size (650 bp) specific for coronatine-producing strains of *P. syringae* pv. *tomato* were amplified from all tested strains. Study of the host range of *P. syringae* pv. *tomato* strains originating from Serbia confirmed tomato as the sole host. The reaction of tomato differential cultivar Ontario 7710 showed that the Serbian strains belonged to races 0 and 1 of *P. syringae* pv. *tomato*.

Key words: Tomato, bacterial speck, identification, races, hosts, *Pseudomonas syringae* pv. *tomato*

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INTRODUCTION

Tomato (*Lycopersicon esculentum*) is one of the major vegetable crops in Serbia. Expansion of tomato production and growing of different tomato varieties contributed to the occurrence of numerous disease problems (Mijatović et al., 1999; Obradović et al., 2004, Milijašević, 2005). Under climatic conditions favorable for disease development, bacterial diseases of tomato pose a serious threat to tomato production during the whole vegetative season (Arsenijević and Jovanović, 1993; Obradović et al., 2004).

During the past two decades, bacterial speck of tomato caused by *Pseudomonas syringae* pv. *tomato* (Okabe) Young, Dye & Wilkie, 1978 has become an important disease of tomato in Serbia (Arsenijević and Jovanović, 1993; Milijašević, 2005). Characteristic symptoms of the disease are necrotic spots surrounded by a chlorotic halo appearing on leaves, as well as scabby lesions on infected fruits. The disease ultimately results in yield loss, mostly due to reduced photosynthetic capacity of infested foliage and from lesions on the fruit that render them unsuitable for the fresh market (Louws et al., 2001; Wilson et al., 2002).

Pseudomonas syringae pv. *tomato* is a seed-borne pathogen and persists in plant debris in soil for a long time. Moreover, epiphytic populations of the pathogen persist on symptomless host plants and weeds and can become infective in favorable weather conditions (Chambers and Merriman, 1975; Schneider and Grogan, 1977; McCarter et al., 1983). In addition to a complex biology of the bacterium, the occurrence of new races of the pathogen raise new problems in selection and breeding of resistant tomato cultivars. Currently, two tomato races of the pathogen (0 and 1) have been described in the world (Lawton and MacNeil, 1986; Bogatsevska, 1989). However, increased selection pressure caused by growing tomato varieties resistant to the predominant race 0 led to development of the new race 1 even on tomato hybrids heterozygous for the *Pto* gene (Buonaurio et al., 1996).

Moreover, data on natural hosts of *P. syringae* pv. *tomato* are scarce. Besides tomato, Oprea and Rafaila (1974, loc. cit. Arsenijević, 1997) reported pepper (*Capsicum annuum*) and eggplant (*Solanum melongena*) as bacterium hosts.

The first objective of this study therefore was to phenotypically characterize the bacterial strains associated with tomato bacterial speck from different regions in Serbia. A further objective was to investigate possible hosts of the bacterium among solanaceous plants. Our final objective was to determine the race structure of the pathogen in Serbia in order to create the basis for screening selection material for resistance and breeding tomato varietes resistant to the pathogen.

MATERIALS AND METHODS

Isolation of bacteria and growth conditions

Bacterial strains were isolated from diseased tomato samples (leaves and fruits) collected from different regions in Serbia during 2002-2004 using standard bacteriological procedures on nutrient sucrose agar (NSA) and King's medium B (KB) (Klement et al., 1990). Typical levan-forming colonies from NSA and fluorescent colonies from KB were selected and transferred to slants of yeast dextrose carbonate medium (YDC) and nutrient agar amended with 2% glycerol (NAG), incubated at 26°C for 24 h, and maintained at 4°C as a working culture for several months. Prior to testing, the bacterial strains were grown on KB or nutrient agar (NA) plates at 26°C to obtain 24-h cultures for all reactions. For long-term preservation, all strains were kept as bacterial suspensions in water, in microvials at room temperature. The Pst CNBP 1323/97 reference strain of *P. syringae* pv. *tomato* (Collection National de Bacteries Phytopathogenes, Angers, France) was used for comparisons in identification of isolated strains. The reference strains DAPP-PG 214 (race 0) and DAPP-PG 213 (race 1) from Italy (kindly supplied by Dr. R. Buonaurio, Dipartimento di Arboricoltura e Protezione delle Piante, Perugia) and DC-84-1 (race 0) and BMG-13 (race 1) from Canada (kindly supplied by Dr. D. Cuppels, Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research, London, Ontario) were used as control strains in race determination tests.

Pathogenicity tests

Tomato plants cv. Saint Pierre and pepper plants cv. California Wonder were grown in a greenhouse in 10-cm pots containing sterile substrate "B medium coarse" (Floragard, Germany) until they reached the five-leaf stage. Inoculum was prepared by growing the investigated strains and reference strains on KB plates at 26°C for 24 h. Bacterial cells were suspended in sterile distilled water and bacterial suspension was adjusted to 10^8 CFU ml^{-1} using McFarland's scale and confirmed by dilution plating on NA medium (Klement et al., 1990). Tomato and pepper plants were sprayed with the bacterial suspensions using a hand-held sprayer until run-off. Control plants were sprayed with sterile distilled water. Additionally, bacterial suspension (diluted to 10^6 CFU ml^{-1}) was infiltrated into tomato and pepper leaves using a medical syringe. Control plants were infiltrated with sterile distilled water.

Following inoculation, the plants were covered with plastic bags for a period of 48 h in order to obtain high humidity and then kept in a climate chamber at 25-27°C, relative humidity of 75-80%, and 12-h daylength for seven days, during which they were observed for symptoms daily.

Pathogenicity of strains was also tested by stab inoculation of young, immature tomato and pepper fruits using a syringe. Control fruits were treated in the same way with water as a negative control and with bacterial suspensions of Pst CNBP 1323/97 reference strain of *P. syringae* pv. *tomato* and a strain of *P. syringae* pv. *syringae* (Arsenijević and Jovanović, 1993). Inoculated fruits were placed in a damp chamber at room temperature and symptoms were observed for seven days.

Additionally, each strain was tested for hypersensitivity (Klement et al., 1990) by infiltrating the bacterial suspension (10^8 CFU ml^{-1}) into the intercostal tissue of tobacco leaves cv. Samsun. Necrosis of the infiltrated area after 24 h was considered a positive hypersensitive reaction (HR).

After appearance of symptoms on tomato plants, the pathogen was reisolated and Koch's postulates were confirmed.

Bacteriological characteristics of the pathogen

Strains were identified using the tests described by Lelliott and Stead (1987) and Schaad et al. (2001): Gram reaction; fluorescence on KB medium; oxidative-fermentative metabolism of glucose (O/F test); levan formation, oxidase activity, potato rot, arginine-dehydrolase activity, tobacco hypersensitivity (LOPAT tests); starch hydrolysis; gelatin liquefaction; aesculin hydrolysis; catalase activity; NH_3 production; nitrate reduction; acid production from sorbitol, mannitol, inositol, erythritol and L-lactate; and the ice nucleation test.

Polymerase chain reaction (PCR)

In order to confirm the identity of coronatine-producing *P. syringae* pv. *tomato* strains isolated from tomato plants, PCR testing was conducted from each isolate and from culture of the reference strain according to the PCR protocol of Bereswill et al. (1994). To prepare template DNA, cultures were grown on NA for 24 h. Whole bacterial cells (without previous DNA extraction) were suspended in sterile distilled water and bacterial suspension was adjusted to 10^8 CFU ml^{-1} (using McFarland's scale and confirmed by dilution plating on NA medium), then diluted to 10^6 CFU ml^{-1} and briefly centrifuged. In conducting PCR, we used primers specific for detection of coronatine–producing pathovars of *Pseudomonas syringae* where the expected PCR products are 650 base pairs (bp) with the following sequence:

Primer 1: 5` GGCGCTCCCTCGCACTT 3`
Primer 2: 5` GGTATTGGCGGGGGTGC 3`

Primers were synthesized by Invitrogen (USA). The PCR reactions were performed in an Eppendorf Master thermocycler. Amplification of DNA was performed in a total volume of 25 µl. All reactions contained: Red Taq PCR Reaction Mix with $MgCl_2$ (Sigma, Germany) (1.5 U Taq DNA polymerase, 10 mM tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 0.2 mM each dNTP), 1 µl of each primer (20 µM), and 1 µl of template DNA. The Pst CNBP 1323/97 reference strain was used as positive control and reaction mix without addition of DNA (blank) was used as negative control. A low-mass DNA ladder (100 bp) was used. The amplification conditions were: initial denaturation at 93°C for 2 min, followed by 37 cycles of denaturation at 93°C for 2 min, annealing at 67°C for 1 min, and extension at 72°C for 2 min. Analysis of PCR products was performed in 1.5% agarose gel (with addition of ethidium bromide to a final concentration of 0.005%) run at 100 V for 40 min in TAE buffer. Gels were observed in a UV transilluminator and photographed.

Race differentiation

All tomato strains were tested for elicitation of bacterial speck symptoms typical for *P. syringae* pv. *tomato* in differential tomato cultivar Ontario 7710, which carries the resistance gene (*Pto*) against race 0 of the pathogen. Races of *P. syringae* pv. *tomato* were identified on the basis of presence or absence of typical disease symptoms on cv. Ontario 7710. The occurrence of bacterial speck symptoms in this differential tomato cultivar represents a compatible reaction between the host and the pathogen and therefore indicates the presence of race 1 of the pathogen (Buonaurio et al., 1996).

Plants of the differential tomato cultivar were inoculated at the four-leaf stage by spraying with bacterial suspension (concentration adjusted to 10^8 CFU ml^{-1} using McFarland's scale) (Klement et al., 1990). Control strains used for race differentiation were: DAPP-PG 214 (race 0), DAPP-PG 213 (race 1), DC-84-1 (race 0), and BMG-13 (race 1), while tomato plants sprayed with water served as a negative control.

Following inoculation, the plants were covered with plastic bags for a period of 48 h in order to obtain high humidity and then kept in a climate chamber at 25°C, relative humidity of 75%, and 12-h daylength for seven days, during which they were observed for symptoms daily.

Host range of the pathogen

The host range of *P. syringae* pv. *tomato* was determined by infiltration of bacterial suspension (adjust-

ed to a final concentration of 10^5 CFU ml^{-1}) into leaf tissue of different plants belonging to the family Solanaceae, followed by monitoring of population dynamics of the pathogen.

The following test plants were used in the study: tomato (*Lycopersicon esculentum* cv. Narvik), pepper (*Capsicum annuum* cv. Palanačka Babura), eggplant (*Solanum melongena*), tobacco (*Nicotiana tabacum* cv. Samsun), nightshade (*Solanum nigrum*), and jimsonweed (*Datura stramonium*).

The potential host plants were inoculated with strain P-107 (previously determined as *P. syringae* pv. *tomato*, race 0). The inoculum was prepared from a culture grown for 24 h on NA plates. The concentration of bacterial suspensions was adjusted using McFarland's scale and confirmed by dilution plating (Klement et al., 1990).

Samples of infiltrated leaf tissue were taken one hour after inoculation and subsequently at 24-h intervals for a period of nine days. Two leaf disks (diameter of 0.56 cm, total area of 1 cm^2) in three replicates were transferred to a mortar and macerated with a pestle in 1 ml of sterile distilled water. Five serial dilutions (1:10) were made out of the suspensions and 100 µl of each dilution was plated onto NA medium. Bacterial colonies were counted after 48-h growth at 26°C and the mean number of bacterial cells per cm^2 was calculated for each test plant.

RESULTS

Disease symptoms

A three-year survey of tomato fields in Serbia resulted in the isolation of numerous bacterial strains, with 30 representative strains selected for further analyses (Table 1).

The first bacterial speck symptoms were recorded in June on field grown tomatoes. Symptoms occurred in all upper parts of the plants. The first signs of the disease appeared as moist, light-green spots on the leaves, which later on became necrotic dark-brown to black in color and surrounded by a chlorotic halo. These spots in time expanded and coalesced, causing necrosis of a larger leaf area, deformations, stunting, and ultimately death of leaves (Fig. 1). Spots also appeared on stems as moist areas with a dark center

Table 1. Strains and races of *Pseudomonas syringae* pv. *tomato*.

Strain code	Locality	Plant organ	Year of isolation	Race
PstBB-1	Šabac	Leaf	2002	1
PstBB-3	Šabac	Leaf	2002	1
PstBB-4	Šabac	Leaf	2002	1
PstBB-6	Šabac	Leaf	2002	1
PstBB-7	Šabac	Leaf	2002	1
PstBB-8	Šabac	Leaf	2002	1
PstBB-9	Šabac	Leaf	2002	1
PstBB-11	Šabac	Leaf	2002	1
Pl-1	Šabac	Leaf	2003	1
Pl-4	Šabac	Leaf	2003	0
Pl-5	Šabac	Leaf	2003	0
P-100	Šabac	Fruit	2003	0
P-102	Šabac	Fruit	2003	0
P-106	Šabac	Fruit	2003	0
P-107	Šabac	Fruit	2003	0
P-108	Šabac	Fruit	2003	0
P-120	Šabac	Fruit	2003	0
P-121	Šabac	Fruit	2003	0
PSP-200	Smederevska Palanka	Leaf	2003	0
PSP-201	Smederevska Palanka	Leaf	2003	0
PSP-202	Smederevska Palanka	Leaf	2003	1
PSP-203	Smederevska Palanka	Leaf	2003	0
Pl-402	Čačak	Leaf	2004	1
Pl-403	Čačak	Leaf	2004	0
Pl-404	Čačak	Leaf	2004	0
Pl-405	Čačak	Leaf	2004	1
PP-412	Čačak	Fruit	2004	0
PP-418	Čačak	Fruit	2004	0
PP-420	Čačak	Fruit	2004	0
PP-421	Čačak	Fruit	2004	0

Fig. 1. Bacterial speck symptoms on tomato leaf – natural infection.

Fig. 2. Bacterial speck symptoms in pathogenicity test seven days after spray-inoculation with *Pseudomonas syringae* pv. *tomato* strain P-100.

which later on became black. Speck symptoms on fruits appeared as small black lesions surrounded by a moist zone that later became larger and surrounded by a yellow or white halo.

Pathogenicity tests

All isolated strains as well as the reference strains caused typical speck symptoms on tomato plants cv. Saint Pierre inoculated by spraying. The first symptoms in the form of moist green spots were observed three days after inoculation. On the fourth day, the spots became small (1-2 mm in diameter) and turned dark-brown with a distinguishable chlorotic halo (Fig. 2). Later on, they expanded and merged, causing necrosis of a larger leaf area. No symptoms were recorded on negative control plants sprayed

Table 2. Bacteriological identification of the pathogen. + = Positive reaction; = Negative reaction; O = Oxidative metabolism of glucose; 1 = King's medium B. 2 = Oxidative-fermentative metabolism of glucose. nt = not tested.

Strains	Investigated strains (30)	Reference strain of *P. syringae* pv. *tomato* (Pst CNBP 1323/97)	Reference strain of *P. syringae* pv. *syringae* Ks-101
Origin	Serbia	France	Serbia
Gram stain	-	-	-
Fluorescence on KB[1]	+	+	+
Glucose (O/F[2]) metabolism	O	O	O
Levan production	+	+	+
Oxidase activity	-	-	-
Potato rot	-	-	-
Arginine-dehydrolase activity	-	-	-
Tobacco hypersensitive reaction	+	+	+
Catalase production	+	+	+
Gelatine hydrolysis	+	+	+
Aesculin hydrolysis	+	+	+
Starch hydrolysis	-	-	nt
NH_3 production	+	+	nt
Nitrate reduction	-	-	
Acid produced from:			
-sorbitol	+	+	+
-mannitol	+	+	+
-inositol	+	+	+
-erythritol	-	-	+
-L-lactate	-	-	+
Ice nucleation	-	-	+

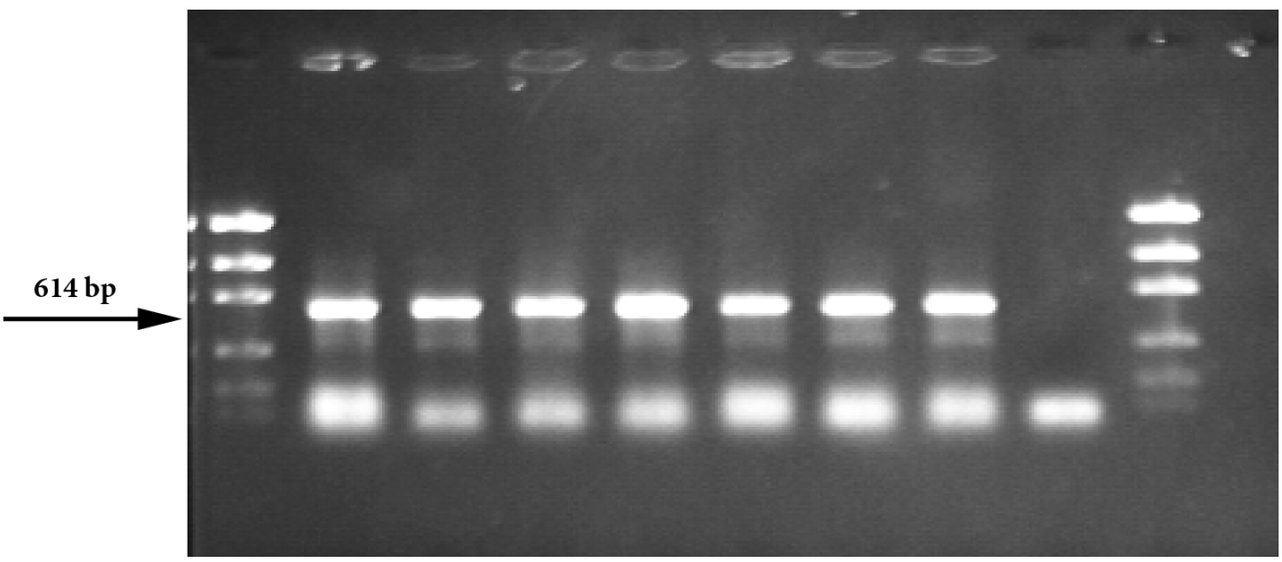

Fig. 3. Amplification of 650 bp DNA fragment. Legend: 1 = Ladder (100 bp), 2 = Strain Pst CNBP 1323/97 (positive control), 3–8 = Tested strains, 9 = Negative control, 10 = Ladder (100 bp).

with water. Bacteria were reisolated from symptomatic leaves and Koch's postulates were confirmed.

No symptoms were recorded on spray-inoculated pepper plants.

All isolated strains as well as the reference strains caused necrotic spots surrounded by a chlorotic halo 4-5 days after infiltration of bacterial suspension (10^6 CFU ml^{-1}) into tomato leaves. In contrast to this, pepper leaf tissue infiltrated with bacterial suspension (10^6 CFU ml^{-1}) of the investigated strains exhibited light green spots after one day which became dark-brown, resembling more a hypersensitive reaction than typical disease symptoms. Plants infiltrated with water had no symptoms.

All tested strains induced small (2-mm) dark brown spots on stab-inoculated immature tomato fruits. These spots grew into larger, scabby lesions with sunken surrounding tissue. In contrast to this, *P. syringae* pv. *syringae* strain Ks-101 (isolated from apricot, collection of plant pathogenic bacteria, Institute of Pesticides and Environmental Protection) caused large black sunken spots covering most of the fruit area, which represented a distinguishing characteristic of *P. syringae* pv. *syringae* compared to *P. syringae* pv. *tomato*. Stab-inoculated pepper fruits exhibited local dark-brown to black necrotic spots as an incompatible reaction, while fruits treated with water had no symptoms.

All strains also induced an HR in tobacco leaves.

Strains and their phenotypic characteristics

The results of conventional bacteriological identification tests are given in Table 2. All strains were Gram-negative, fluorescent on KB medium, and metabolized glucose oxidatively. Based on these characteristics, the isolated strains were classified as fluorescent pseudomonads.

The investigated strains formed the levan type of colonies on NSA, were oxidase- and arginine-dehydrolase-negative, did not produce pectolytic enzymes (were potato rot-negative), and induced an HR in tobacco leaves. According to the results of LOPAT tests, the investigated strains belonged to the Ia *Pseudomonas* group.

Other biochemical and physiological characteristics of all strains were as follows: catalase-positive; gelatin and aesculin hydrolysis-positive; starch hydrolysis-negative; NH_3 production-positive; nitrate reduction-negative; and acid production from sorbitol, mannitol, and inositol-positive.

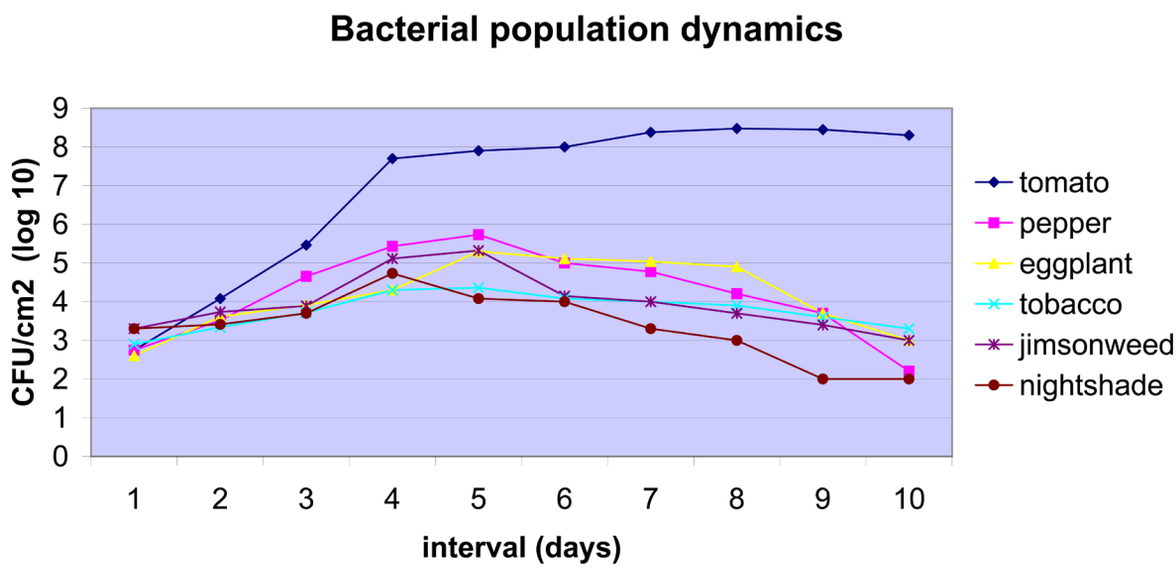

Fig. 4. Bacterial population dynamics.

The investigated strains, as well as the reference strain of *P. syringae* pv. *tomato*, did not use erythritol or L-lactate as a carbon source, and the ice nucleation test was negative. On the other hand, *P. syringae* pv. *syringae* strain Ks-101 metabolized erythritol and L-lactate and was positive in the ice nucleation test. Based on the results of pathogenicity, biochemical, and physiological tests, all strains isolated from diseased tomato plants were identified as *P. syringae* pv. *tomato*.

Polymerase chain reaction (PCR)

The identity of strains isolated from diseased tomato plants was confirmed using the PCR protocol and primer set designed by Bereswill et al. (1994). Polymerase chain reaction products of expected size (650 bp) specific for coronatine-producing strains of *P. syringae* pv. *tomato* were amplified from all investigated strains previously identified by conventional methods as *P. syringae* pv. *tomato*, as well as from the reference strain Pst CNBP 1323/97, while the blank control was negative (Fig. 3).

Characterization of races

The reaction of tomato differential cultivar Ontario 7710 showed that the Serbian strains of *P. syringae* pv. *tomato* belonged to both known races of the pathogen (Table 3). Strains that did not produce any symptoms on differential tomato cultivar Ontario 7710 seven days after inoculation, as well as race 0 reference strains DAPP-PG 214 from Italy and DC-84-1 from Canada, were designated as race 0 of the pathogen. The other group of strains which induced typical bacterial speck symptoms (compatible reaction) on differential tomato cultivar Ontario 7710 identical to those induced by race 1 reference strains DAPP-PG 213 from Italy and BMG-13 from Canada belonged to race 1 of the pathogen. Based on these results, all tomato strains were classified as belonging to races 0 and 1 (Table 1).

Host range of the pathogen

In the form of moist green spots, the first symptoms on tomato leaves inoculated by infiltration of bacterial suspension were recorded three days after inoculation. Two days later, the spots became dark-brown and surrounded by a chlorotic halo resembling natural infection symptoms. Infiltrated pepper leaves exhibited light-green spots 24 h after inoculation, as was recorded in pathogenicity tests. Three days later, leaf tissue became necrotic, as in a hypersensitive reaction. Similar HR-like changes were recorded in infiltrated nightshade, jimsonweed, tobacco, and eggplant leaf tissue.

Monitoring of bacterial population dynamics (Fig. 4) showed that bacterial populations in leaf tissues of pepper, eggplant, tobacco, nightshade, and jimsonweed increased for the first three days and decreased afterwards. In contrast to this, the bacterial population in infiltrated tomato leaf tissue increased continually up until the ninth day, when typical speck symptoms appeared and confirmed a compatible reaction between the pathogen and the host.

DISCUSSION

Favorable climatic conditions for disease development and limited possibilities of controlling bacterial diseases of tomato have contributed to the frequent occurrence of *P. syringae*. pv. *tomato* infection during the past 20 years in Serbia (Arsenijević and Jovanović, 1993; Milijašević, 2005). Bacterial speck was detected in several major tomato-growing regions in Serbia (Table 1) during a three-year period. The first bacterial speck symptoms were recorded in June on field-grown tomatoes. Higher disease incidence was recorded in fields after heavy rainfall. Fruit size and quality were reduced compared with those from non-infected fields.

The results of conventional identification of strains using bacteriological methods indicated that *P. syringae* pv. *tomato* was associated with bacterial speck symptoms in tomato field crops. The investigated strains showed a high level of pathogenicity, inducing symptoms similar to those in natural infection of tomato plants seven days after spray-inoculation (Fig. 2). On the other hand, no symptoms were recorded on spray-inoculated pepper plants. All isolated strains caused necrotic spots surrounded by a chlorotic halo following inoculation by infiltration of bacterial suspension (10^6 CFU ml^{-1}) into tomato leaves, while infiltrated pepper leaf tissue exhibited a hypersensitive reaction.

All tested strains induced small dark-brown spots on stab-inoculated immature tomato fruits, which grew into larger scabby lesions with sunken surrounding tissue. In contrast to this, the *P. syringae* pv. *syringae* strain caused large black sunken spots covering most of the fruit area, which represented a distinguishing characteristic of *P. syringae* pv. *syringae* compared to *P. syringae* pv. *tomato*. Stab-inoculated pepper friuts exhibited local dark-brown to black necrotic spots as an incompatible reaction. Similar results of pathogenicity tests on tomato and pepper leaves and immature tomato and pepper fruits were reported by Arsenijević and Jovanović (1993). Using different inoculation methods, these authors concluded that the best method of testing the pathogenicity of *P. syringae* pv. *tomato* was inoculation of tomato leaves by spraying, since this method ensured reproduction of typical disease symptoms, i.e., small dark-brown spots surrounded by a chlorotic halo. The results of our study confirmed the conclusion of these authors. All strains also induced an HR in tobacco leaves, which is an important characteristic of fluorescent pseudomonads belonging to group Ia.

In spite of the fact that Oprea and Rafaila (1974, loc. cit. Arsenijević, 1997) reported pepper as a natural host of *P. syringae* pv. *tomato*, results of the pathogenicity tests conducted in this study and host range experiments in which population dynamics was monitored showed that the investigated strains caused atypical necrosis similar to a hypersensitive reaction in pepper tissue.

Similar HR-like necrosis was recorded in leaf tissue of eggplant, tobacco, jimsonweed, and nightshade. The first symptoms on tomato leaves inoculated by infiltration of bacterial suspension were recorded three days after inoculation. Two days later, spots became dark-brown and surrounded by a chlorotic halo, thereby resembling natural infection symptoms.

Monitoring of bacterial population dynamics (Fig. 4) showed that bacterial populations in leaf tissues of pepper, eggplant, tobacco, nightshade, and jimsonweed increased for the first three days and decreased afterwards. In contrast to this, the bacterial population in infiltrated tomato leaf tissue increased continually up until the ninth day, when typical speck symptoms appeared and confirmed a compatible reaction between the pathogen and the host.

Based on pathogenicity tests and population

dynamics in different solanaceous plants, only tomato was confirmed as a host plant of *P. syringae* pv. *tomato*.

Products of expected size (650 bp) specific for coronatine-producing strains of *P. syringae* pv. *tomato* were amplified from all investigated strains, as well as from the Pst CNBP 1323/97 reference strain, while the blank control was negative (Fig. 3). Thus, the results of molecular identification using the polymerase chain reaction as described by Berreswill et al. (1994) confirmed that strains previously identified by conventional methods as *P. syringae* pv. *tomato* belong to coronatine-producing strains of this bacterium. Although recent work of Zaccardelli et al. (2003) pointed to the existence of *P. syringae* pv. *tomato* strains which do not have the gene responsible for production of the phytotoxin coronatine (Cor^- strains), these strains were not detected in our study.

The reaction of the tomato differential cultivar Ontario 7710 showed that the Serbian strains of *P. syringae* pv. *tomato* belong to races 0 and 1 of the pathogen (Table 3). Out of 30 investigated *P. syringae* pv. *tomato* strains, 18 strains did not induce symptoms on the Ontario 7710 differential cultivar and were therefore assigned to race 0, in contrast to the remaining 12 strains, which belonged to race 1 of the pathogen (Table 3). The presence of both races of the pathogen was previously reported in Canada (Lawton and MacNeil, 1986) and a few years later in Bulgaria (Bogatsevska, 1989). Moreover, Buonaurio et al. (1996) reported the occurrence of race 1 in tomato hybrids heterozygous for the *Pto* gene in Italy.

The arisal of new races of pathogens after introduction of resistant genotypes in crop production is a well known phenomenon in complex host-pathogen interactions (Mew et al., 1992). Moreover, shifts in races or in the competitive advantage of one race over another have already been reported (Jones et al., 1998; Pohronezny et al., 1992). One way to explain the presence of both races of the pathogen in Serbia is to postulate the possible existence of race 1 in the natural bacterial population. Another feasible option is that increased selection pressure occurred with the introduction and growing of new imported tomato varieties and hybrids. Since tomato is intensively grown on the Balkan Peninsula and in Mediterranean countries and considering the seed-borne nature of the pathogen, we cannot exclude the possibility of introduction of new races as a consequence of uncontrolled import of infected tomato seeds and fresh fruit exchange between neighboring countries where race 1 has already been reported.

The results of our study showed that race 1 was prevalent on tomatoes in Šabac during 2002. However, conclusive results indicated the predominant presence of race 0 elsewhere in Serbia. This fact emphasizes the importance of knowing the race structure of *P. syringae* pv. *tomato* in different tomato-growing regions of Serbia so as to ensure the possibility of choosing tomato genotypes resistant to the prevalent race of the bacterium. These results also indicate the necessity of testing the susceptibility of genotypes commonly grown in Serbia against both races of the bacterium and the need to discover new sources of resistance in order to counter new races of the pathogen. Stockinger and Walling (1994) found new resistance genes (*Pto*3 and *Pto*4) in *Lycopersicon hirsutum* var. *glabratum* capable of conferring resistance to races 0 and 1 of the bacterium (respectively). Introduction of these resistance genes into commercial tomato genotypes would significantly improve control of *P. syringae* pv. *tomato.*

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РАСЕ И ДОМАЋИНИ *PSEUDOMONAS SYRINGAE* PV. *TOMATO* У СРБИЈИ

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Током последњих неколико година, уочена је учестала појава бактериозне пегавости лишћа и краставости плодова парадајза у неколико рејона гајења ове повртарске културе у Србији. Трогодишњим прегледом (2002-2004) оболелих биљака парадајза гајених у пољу сакупљен је и изолован већи број сојева бактерија од којих је за даља проучавања одабрано 30 репрезентативних. На основу резултата проучавања патогених и биохемијско-физиолошких одлика, утврђено је да

сви сојеви изоловани из узорака оболелих биљака парадајза припадају бактерији *Pseudomonas syringae* pv. *tomato*. Идентитет сојева потврђен је ланчаном реакцијом полимеразе (PCR), с обзиром да су из свих проучаваних сојева амплификовани PCR продукти очекиване величине (650 bp) специфични за сојеве *P. syringae* pv. *tomato* који производе фитотоксин коронатин. Проучавањем спектра домаћина сојева *P. syringae* pv. *tomato* пореклом из Србије доказано је да је парадајз једини домаћин ове бактерије. Разлике испољене у реакцији диференцијалне сорте парадајза Ontario 7710 показале су да наши сојеви припадају расама 0 и 1 ове бактерије.