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CHARACTERIZATION OF *Pseudomonas syringae* pv. *morsprunorum* ORIGINATING FROM SWEET CHERRY AND PLUM IN SERBIA

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Pseudomonas strains originating from symptomatic (bacterial spot) leaf tissues of sweet cherry (Topola, Šumadija) and plum (Krušedol Selo, Srem) were isolated during 2016 and 2020, respectively. Based on the findings yielded by classical microbiological methods, LOPAT (+---+), GATTa (--++) and pathogenicity tests performed on detached fruitlets (sweet and sour cherry) and pods (bean pods), all strains were confirmed to belong to *P. syringae* pv. *morsprunorum*. The detection of *cfl* gene allowed strains that belong to race 1 to be identified. The DNA fingerprinting patterns obtained with four rep-PCR (BOX and ERIC), RAPD-PCR (M13), and IS50-PCR (IS50) methods revealed that the seven tested sweet cherry and plum *P. s.* pv. *morsprunorum* strains, as well as

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comparative KBNS71 and the reference strain CFBP 2119, were genetically heterogeneous. Conversely, MLSA based on the four-gene-based scheme (*gapA*, *gltA*, *gyrB*, and *rpoD*) indicated genetic homogeneity among all tested Serbian sweet cherry and plum strains, as well as *P. s.* pv. *morsprunorum* race 1 strains from the NCBI. Although the MLSA findings indicate that the sweet cherry and plum strains used in this study are 100% identical, as they might have different virulence genes, genome sequencing should be performed to eventually find the strain sub-clades based on the host.

Keywords: bacterial leaf spot, stone fruits, diversity, DNA fingerprinting, MLSA

INTRODUCTION

Cultivation of stone fruit such as cherries, and especially plums, has always been one of the most important aspects of Serbian agriculture. Generally, *Pseudomonas syringae* belonging to the fluorescent group are considered as the most destructive bacterial pathogens of stone fruit, and can be detrimental to production, especially in young orchards and plantations (ILIČIĆ *et al.*, 2018; 2021a; 2021b). According to the available data, *Pseudomonas syringae* pvs. *syringae* and *morsprunorum* are the most significant contributors to production losses (GAVRILOVIĆ *et al*., 2013; BALAŽ *et al*., 2016; ILIČIĆ, 2016; NOSRATNEZHAD *et al*., 2018; GIOVANARDI *et al*., 2018; KHEZRI and MOHAMMADI, 2018; ILIČIĆ *et al.*, 2021a; 2021b). These pathovars typically cause symptoms such as wood cankers on trees, necrotic spots on leaves and fruits, and premature defoliation. The host range of the *P. s.* pv. *morsprunorum* is limited to stone fruit species, namely cherry, wild cherry, apricot, sour cherry, peach, almond, plum, Japanese plum, and Japanese apricot (SCORTICHINI *et al.*, 1995; LEE *et al.*, 2012; ILIČIĆ *et al.*, 2021a; 2021b).

Identification of species from the *P. syringae* group requires a combination of classical microbiological methods and complex molecular analyses, including comparison of DNA fingerprinting patterns, sequencing of housekeeping genes, etc. (KAŁUŻNA *et al.*, 2016). Among the molecular methods, rep-PCR and MLSA have been proven as particularly valuable in the assessment of phylogenetic relations among *P. syringae* isolates (MARTIN-SANZ *et al.*, 2013; GIOVANARDI *et al.*, 2018). Thus far, 13 phylogroups have been described within the *P. syringae* complex based on the multilocus sequence typing (MLST) (SARKAR and GUTTMAN, 2004; PARKINSON *et al*., 2011; BERGE *et al.*, 2014). Available data further indicate that, based on colony structure, biochemical, serological, and pathological characteristics, *Pseudomonas s.* pv. *morsprunorum* can be segregated into two highly homogeneous races, denoted as race 1 and race 2 (FREIGOUN and CROSSE, 1975; VICENTE *et al.*, 2004). Based on DNA hybridization, these races are two distinct organisms that belong to the genomospecies 2 and 3, respectively, while *P. s.* pv. *syringae* belongs to the genomospecies 1 (GARDAN *et al.*, 1999). The genetic profiles generated by rep-PCR, especially BOX-PCR, have been useful in distinguishing *P. s.* pv. *morsprunorum* isolates to the race level (VICENTE and ROBERTS, 2007; GILBERT *et al*., 2009; LEE *et al.*, 2012; NOSRATNEZHAD *et al.*, 2018). As most of the *P. s.* pv. *morsprunorum* race 1 strains produce the phytotoxin coronatine, coronatine synthesis (*cfl*) gene detection is useful for distinguishing race

1 from race 2 as well as from *P. s.* pv. *syringae* (BERESWILL *et al.*, 1994; NOSRATNEZHAD *et al.*, 2018).

Guided by this evidence, the aim of the present work was to characterize *P. s.* pv. *morsprunorum* strains originating from sweet cherry (*Prunus avium*) and plum (*Prunus domestica*) using different molecular and classical microbiological techniques to examine relevant genetic and phenotypic features of the bacterial population currently present on these stone fruit species in Serbia.

MATERIALS AND METHODS

P. syringae pv. morsprunorum strains

Three Serbian *P. s.* pv. *morsprunorum* strains originating from diseased leaves of sweet cherry with bacterial spot symptoms grown in Topola in 2016 (strains coded as Pm5, Pm8, and Pm9) as well as four strains originating from diseased leaves of plum with bacterial spot symptoms grown in Krušedol Selo in 2020 (strains coded as Pm21, Pm22, Pm26, and Pm27) were used in this study. All strains were kept at -20 °C in Luria broth (LB) medium with addition of 20% glycerol until required for analyses. Reference strain CFBP 2119 (*P. s.* pv. *morsprunorum*, race 1) isolated from *Prunus cerasus* in France (Collection Française des Bactéries Phytopathogènes, INRA, Angers, France) was used in all tests. Additionally, *P. s.* pv. *morsprunorum* race 1 strain KBNS71, isolated from sweet cherry in Selenča (Serbia) in 2012 (BALAŽ *et al.*, 2016), was used as a comparative strain.

Genetic Characterization

Genomic DNA extraction

Genomic DNA was extracted from seven tested strains—three Serbian sweet cherry and four Serbian plum strains (Pm5, Pm8, Pm9, Pm21, Pm22, Pm26, and Pm27)—one comparative *P. s.* pv. *morsprunorum* strain (KBNS71) and the reference *P. s.* pv. *morsprunorum* strain CFBP 2119 according to the hexadecyltrimethylammonium bromide (CTAB) procedure proposed by LE MARREC *et al.* (2000) and modified as described by POPOVIĆ *et al.* (2019). Before the extraction, all strains were grown in Nutrient Sucrose Agar (NSA) medium for 48 hours at 25°C. Single bacterial colonies of each strain were re-suspended in 500 mL of sterile distilled water and centrifuged at 10,000×g for 10 min. The resulting pellet was washed in a mixture containing TE buffer (50 mM Tris, pH 8, 1 mM EDTA), 10% (w/v) sodium dodecyl sulfate (SDS), and proteinase K (20 mg/mL) and was subsequently incubated at 37°C for 30 min. Thereafter, samples were treated with 5M NaCl and were incubated for 20 min at 65°C after the addition of 3% CTAB. Chloroform was used for purifying the obtained DNA. The mixture containing 750 mL of chloroform was centrifuged at 10,000×g for 15 min. The obtained upper (aqueous) phase was transferred to new tubes, washed with ice-cold isopropanol, and centrifugated at 10,000×g for 15 min. Finally, the obtained pellet was washed using 96% ice-cold ethanol before being centrifugated again at 10,000×g for 10 min. DNA was dried at room temperature and dissolved in 50 μL of TE buffer before storage at -20°C.

Preparation of PCR mixture

Mixtures required for all PCR were prepared using single PCR reagents procured from KAPA Taq PCR Kit (KAPA Biosystems, USA). Amplifications were performed in a total reaction volume of 25 µL containing the following components: (i) 18.8 µL of ultrapure DNase/RNase free water, (ii) 2.5 µL of KAPA Taq Buffer A (×10), (iii) 0.5 µL of KAPA dNTP Mix (10 mM), (iv) 0.2 µL of KAPA Taq polymerase (5U/µL), (v) 1 µL of each of the forward/reverse primers (10 µM), and (vi) 1 µL of the total sample DNA.

Detection of coronatine synthesis (cfl) gene

PCR amplification for *cfl* gene detection was performed using Primer1/Primer2 primer pair (BERESWILL *et al*., 1994), as shown in Table 1. The protocol consisted of 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, with a final extension step at 72°C for 10 min.

Genetic profiling

Three genetic profiling methods—repetitive element palindromic PCR (rep-PCR), Random Amplified Polymorphic DNA (RAPD-PCR), and Insertion Sequences 50 (IS50-PCR)—were adopted to evaluate genetic diversity among seven tested *P. s.* pv. *morsprunorum* strains, one comparative (KBNS71), and one reference strain (CFBP 2119). Rep-PCR was performed using BOXA1R (BOX-PCR) and ERIC1R/ERIC2 (ERIC-PCR) primers, corresponding to the BOXA1R subunit of the BOX element of *Streptococcus pneumonia* and enterobacterial repetitive intergenic consensus sequences, respectively, as well as the $(GTG)_5$ primer for $(GTG)_5$-PCR (LOUWS *et al*., 1994; VERSALOVIĆ *et al*., 1994). RAPD-PCR was performed with primer M13 (M13-PCR) according to the procedure described by HUEY and HALL (1989), while primer IS50 was used for IS50-PCR, in accordance with the methodology described by WEINGART and VÖLKSCH (1997). All primer sequences are listed in Table 1.

The following steps were performed for all PCR amplifications: initial denaturation at 95°C for 7 min (BOX- and ERIC-PCR), 6 min [$(GTG)_5$-PCR], 3 min (IS50-PCR) or at 94°C for 2 min (M13-PCR); followed by 30 [BOX-, ERIC-, and $(GTG)_5$-PCR] cycles, 35 cycles (IS50-PCR) or 40 cycles (M13-PCR) of denaturation at 94°C for 1 min; annealing at 52°C (BOX- and ERIC-PCR), 40°C [$(GTG)_5$-PCR], and 38°C (IS50-PCR) for 1 min or 42°C for 20 s (M13-PCR), and polymerization at 65°C for 8 min [BOX-, ERIC-, and $(GTG)_5$-PCR] or at 72°C for 2 min (M13-PCR) and 3.5 min (IS50-PCR). The final elongation step was performed at 65°C [BOX-, ERIC-, and $(GTG)_5$-PCR] for 16 min or at 72°C for 10 min (M13- and IS50-PCR) (HUEY and HALL, 1989; LOUWS *et al*., 1994; VERSALOVIĆ *et al*., 1994; WEINGART and VÖLKSCH, 1997).

DNA fingerprinting patterns, obtained after amplification of strain DNA with each of the five used primers, were compared using the PHYLogeny Inference Package (PHYLIP) software version 3.698 (FELSENSTEIN, 2004). Unweighted pair group method with arithmetic mean (UPGMA) phylogenetic tree was performed using the NJPLOT program version 2.3 (PERRIÈRE and GOUY, 1996). In the resulting UPGMA tree, each cluster corresponded to one DNA fingerprinting pattern.

Table 1. Primers used in this study

Primer/Locus name	Primer sequence (5'-3')
Primers for cfl gene detection	
Primer 1	GGCGCTCCCTCGCACTT
Primer 2	GGTATTGGCGGGGGTGC
Primers for genetic profiling	
BOXAIR	CTACGGCAAGGCGACGCTGACG
ERIC1R	ATGTAAGCTCCTGGGGATTCAC
ERIC2	AAGTAAGTGACTGGGGTGAGCG
GTG5	GTGGTGGTGGTGGTG
M13	GAGGGTGGCGGTTCT
IS50	CAGGACGCTACTTGTGT
Primers for MLST	
gltA (Forward)	AGTTGATCATCGAGGGCGCWGCC
gltA (Reverse)	TGATCGGTTTGATCTCGCACGG
gapA (Forward)	CGCCATYCGCAACCCG
gapA (Reverse)	CCCAYTCGTTGTCGTACCA
gyrB (Forward)	MGGCGGYAAGTTCGATGACAAYTC
gyrB (Reverse)	TRATBKCAGTCARACCTTCRCGSGC
rpoD (Forward)	AAGGCGARATCGAAATCGCCAAGCG
rpoD (Reverse)	GGAACWKGCGCAGGAGTCGGCACG

Multilocus sequence analysis (MLSA)

Multilocus sequence analysis (MLSA) was conducted based on the partial coding sequences of four housekeeping genes, whereby protocol described by HWANG *et al*. (2005) was adopted for *gapA* (glyceraldehyde-3-phosphate dehydrogenase A), *gyrB* (DNA gyrase subunit B), and *rpoD* (RNA polymerase sigma factor), and that proposed by SARKAR and GUTTMAN (2004) was utilized for *gltA* (citrate synthase). The resulting primer sequences are presented in Table 1. The following steps were conducted for all PCR amplifications: initial denaturation at 94°C for 3 min; followed by 30 cycles of denaturation at 94°C for 2 min; annealing at 54°C (*gapA*), 56°C (*gltA*), 62°C (*gyrB*) or 63°C (*rpoD*) for 1 min; extension at 72°C for 1 min; and a final extension step at 72°C for 10 min. Before sequencing at the Eurofins Genomics sequencing service (Hamburg, Germany), PCR products were purified using the QIAquick PCR Purification and Gel Extraction Kits from QIAGEN GmbH (Hilden, Germany). Sequences were checked for quality and one sequence representing each host/locality/year of isolation was deposited into the National Center for Biotechnology Information (NCBI) database under the following accession numbers: *gapA* (Pm5 − OP535446 and Pm26 − OP535447), *gltA* (Pm5 − OP535448 and Pm26 − OP535449), *gyrB* (Pm5 − OP535450 and Pm26 − OP535451), and *rpoD* (Pm5 − OP535452 and Pm26 − OP535453).

Phylogenetic analysis was performed to compare three Serbian sweet cherry and four plum Serbian *P. s.* pv. *morsprunorum* strains in focus of this study with one comparative Serbian *P. s.* pv. *morsprunorum* strain obtained from sweet cherry in 2012 (KBNS71) and seven additional *P. syringae* (*amygdali*) pv. *morsprunorum* strains isolated from different hosts and originating from different countries [CFBP 2116 (race 1), CFBP 3840 (race 1), R15244, IO 25b (race 1), IO 732 (race 2), and CFBP 6411 (race 2)], as shown in Table 2.

Table 2. NCBI accession numbers of comparative strains used for phylogenetic analysis

Species	Strain code	Host	Country	*gapA*	*gltA*	*gyrB*	*rpoD*
P. s. pv. *morsprunorum*	CFBP 2116 (race 1)	sour cherry	France	LT985192	LT985192	LT985192	LT985192
P. s. pv. *morsprunorum*	CFBP 3840 (race 1)	sweet cherry	France	LT963409	LT963409	LT963409	LT963409
P. a. pv. *morsprunorum*	R15244	sweet cherry	UK	CP026558	CP026558	CP026558	CP026558
P. s. pv. *morsprunorum*	KBNS71 (race 1)	sweet cherry	Serbia	KR051326	KR051298	KR051270	KR051354
P. s. pv. *morsprunorum*	IO 25b (race 1)	-	Poland	HG000149	HG000084	HG000214	HG000019
P. s. pv. *morsprunorum*	IO 732 (race 2)	-	Poland	HG000197	HG000132	HG000262	HG000067
P. s. pv. *morsprunorum*	CFBP 6411 (race 2)	sweet cherry	UK	NZ_LT963408	NZ_LT963408	LT963408	NZ_LT963408
P. s. pv. *syringae*	RE3	sweet cherry	Serbia	MT543299	MT543297	MT543295	MT543292
P. s. pv. *syringae*	RE05	sweet cherry	Serbia	MT543298	MT543296	MT543293	MT543291
P. s. pv. *syringae*	NCPPB 1652	Pea	South Africa	HE604359	HE604374	HE604389	HE604404
P. cerasi	PL963	sweet cherry	Poland	LT963395	LT963395	LT963395	NZ_LT963395
P. s. pv. *avii*	CFBP3846	sweet cherry	France	LT963402	NZLIIJ01000301	JN190425	JN185892
P. s. pv. *persicae*	NCPPB 2254	Peach	France	NZ_ODAM01000035	LAZV01000082	LAZV01000085	NZLAZV01000167
P. azotoformans	P45A	-	Canada	NZ_CP041236	NZ_CP041236	NZ_CP041236	NZ_CP041236

Additionally, different stone fruit pathogens, *P. s.* pvs. *syringae* (RE3, RE05, and NCPPB1652), *avii* (CFBP 3846), and *persicae* (NCPPB 2254), as well as *P. cerasi* (PL963), were included in the phylogenetic analysis. Sequences of all comparative strains were retrieved from the NCBI database (Table 2). *Pseudomonas azotoformans* strain P45A (Accession No.

NZ_CP041236), also sourced from the NCBI database, was used as an outgroup. Before constructing the neighbor-joining (NJ) phylogenetic tree, DNA sequences of all four genes (*gapA*, *gltA*, *gyrB*, and *rpoD*) were aligned using the ClustalW Multiple Alignment function implemented in BioEdit v. 7.0.5 program and were trimmed to the same size (*gapA* – 529 nt, *gltA* – 526 nt, *gyrB* – 569 nt, and *rpoD* – 482 nt). Genetic distances were computed using the Kimura two-parameter nucleotide substitution model (KIMURA, 1980). The NJ tree was constructed for each gene separately and with concatenated sequences (2106 nt) of all four genes.

Gel visualization

The obtained PCR products were mixed with DNA Gel Loading Dye 6X (Thermo Scientific) and were visually inspected for the presence of a band with the expected size [*cfl* (650 bp), *gapA* (634 bp), *gyrB* (610 bp), *rpoD* (521 bp), and *gltA* (556 bp)] in relation to the FastGene 100 bp DNA Marker (Nippon Genetics Europe). For this purpose, electrophoresis was performed for 90 min under a constant voltage of 90 V on 1% agarose gels made in 0.5× TBE buffer, stained with ethidium bromide (0.5 µg mL^{-1}). For rep-, IS50-, and RAPD-PCR, electrophoresis was performed on 2% agarose gel for 2.5 h at 90 V. The position of the obtained DNA fingerprinting patterns was examined in relation to the 1 kb GeneRuler DNA Ladder (Thermo Scientific).

Phenotypic Characterization

Pathogenicity

Pathogenicity tests were performed on immature sweet (*P. avium*) and sour cherry (*P. cerasus*) fruitlets and green bean (*Phaseolus vulgaris*) pods. For this purpose, strains grown on NSA at 26ºC for 48 hours were suspended in sterile distilled water (SDW) to the final concentration of 10^8 CFU mL^{-1}. Sweet and sour cherry fruitlets were inoculated using methods described by KALUZNA and SOBICZEWSKI (2009), while green bean inoculation tests were based on the method developed by ERCOLANI *et al.* (1974).

In all tests, SDW and reference strain served as negative and positive controls, respectively. Prior to performing the assessments, inoculated fruitlets and pods were kept in plastic boxes kept at constant temperature (24±1ºC) and high relative humidity.

Physiological and biochemical tests

Tested sweet cherry and plum strains were examined for Gram reaction, presence of catalase, production of fluorescent pigment on King's B medium under UV light, and oxidative-fermentative metabolism of glucose. They were also subjected to LOPAT tests (production of levan on NSA, presence of oxidase, potato soft rot, presence of arginine dihydrolase, and hypersensitivity reaction (HR) on tobacco leaves), in accordance with the protocol described by LELLIOTT *et al*. (1966). In addition, GATTa tests (gelatin liquefaction, aesculin hydrolysis, tyrosinase activity and utilization of tartrate) were performed following the procedures proposed by LATORRE and JONES (1979).

Sensitivity to ten antibiotics—amikacin 30 ppm, erythromycin 15 ppm, penicillin 6 ppm, kanamycin 30 ppm, vancomycin 30 ppm, chloramphenicol 30 ppm, neomycin 30 ppm,

gentamycin 30 ppm, tetracycline 30 ppm, streptomycin (at 10, 25, 300 and 500 ppm)—was determined using antibiogram tablets (Torlak, Belgrade, Serbia). For this purpose, antibiotic discs were placed on nutrient agar (NA) medium previously inoculated with a pure 48 h culture of the examined strains adjusted to 10^6 CFU ml^{-1}. After incubation at 26 °C for 24 hours, Petri plates were checked for the presence of inhibition zones.

RESULTS AND DISCUSSION

Bacterial leaf spot disease was observed on sweet cherry (locality Topola, Šumadija) and plum (locality Krušedol Selo, Srem) during May 2016 and June 2020, respectively. Initial symptoms appeared as water-soaked, irregularly shaped spots on leaves, and as the disease developed, spots enlarged, turned dark brown and dropped out giving a shot-hole appearance, as shown in Figure 1. *Pseudomonas syringae* pvs. are usually isolated from leaves exhibiting such symptoms on nutrient media. As a part of this investigation, bacterium *P. s.* pv. *morsprunorum* was isolated in both sweet cherry and plum. These findings are in accordance with previous reports of similar bacterial leaf spot disease symptoms on stone fruit on several locations in Serbia, which were ascribed to bacterium *P. s.* pv. *morsprunorum* race 1 (BALAŽ *et al*., 2016; ILIČIĆ, 2016; ILIČIĆ *et al*., 2018; 2019). This pathovar was also determined as a causal agent of severe bud necrosis in both cherry and plum in Serbia by GAVRILOVIĆ *et al.* (2013). According to ILIČIĆ *et al.* (2022) *Pseudomonas cerasi* is another causal agent of bacterial leaf spot affecting wild cherry in Serbia.

Figure 1. Bacterial leaf spot on (a) sweet cherry at the Topola locality and (b) plum at the Krušedol selo locality (photo: Iličić, R.)

Genetic Characterization

The *cfl* gene was detected in all tested strains (three pertaining to sweet cherry and four to plum) after amplification of an expected 650 bp fragment specific to the *P. s.* pv.

morsprunorum race 1, as well as in the reference (CFBP 2119) and comparative (KBNS71) strains. According to GIOVANARDI *et al.* (2018), the detection of the *cfl* gene involved in coronatine production can be used for *P. s. morsprunorum* race 1 identification. On the other hand, KAŁUŻNA *et al.* (2016) noted that the determination of gene encoding for toxin production is not reliable for identification of *P. syringae* pvs. strains, since some strains do not have the ability to produce coronatine, and thus *cfl* presence/absence cannot be the only criterion for strain classification. Nonetheless, positive results for all Serbian *P. s.* pv. *morsprunorum* strains were obtained in the present study.

Based on the DNA fingerprinting patterns obtained with four rep- (BOX and ERIC), RAPD- (M13), and IS50-PCR (IS50) primers, Serbian sweet cherry and plum strains—both tested (Pm5, Pm8, Pm9, Pm21, Pm22, Pm26, and Pm27) and comparative (KBNS71)—and the reference strain CFBP 2119 were shown to be genetically heterogeneous (Figure 2). However, $(GTG)_5$-PCR generated identical patterns only for the seven tested Serbian sweet cherry and plum strains. Patterns obtained with primer IS50 showed the lowest complexity in terms of the number of generated bands (three to four), while ERIC and M13 exhibited the most complex and the most visually perceptible patterns with up to nine and eight bands, respectively. Considering that no differences within each group of strains, based on host, locality, and year of isolation, were observed irrespective of the adopted methods, one strain from each group (i.e., sweet cherry strain Pm5 and plum strain Pm26) was randomly selected as an example of DNA fingerprinting patterns depicted in Figure 2, while UPGMA phylogenetic trees for each amplified primer are shown in Figure 3. Based on the presented trees, BOX-, ERIC-, M13-, and IS50-PCR separated strains into four groups, while three groups were generated by $(GTG)_5$-PCR, which thus had inferior discriminatory power. These findings concur with the available evidence, indicating that, among the DNA-fingerprinting methods, rep-PCR (BOX-, ERIC-, and REP-PCR) and IS50-PCR tend to exhibit greater potential for successful differentiation of pathogens causing stone fruit bacterial cankers (BULTREYS and KALUZNA, 2010). Nonetheless, RADEMAKER *et al.* (2000) recommended combined use of several rep-PCR methods (BOX, ERIC, and REP) to obtain a more consistent clustering of strains. However, more recently, VICENTE and ROBERTS (2007) opined that even independent use of BOX, ERIC, and REP can provide adequate differentiation between *P. s.* pv. *morsprunorum* races 1 and 2, as well as their separation from the closely related *P. s.* pv. *syringae* strains. Based on the results obtained in the present study, similar discriminatory capacity was observed for all five DNA fingerprinting primers employed for this purpose. However, BOX-, ERIC-, M13-, and IS50-PCR can be singled out as superior to $(GTG)_5$-PCR. In their study, GILBERT *et al.* (2009) tested several *P. s.* pv. *morsprunorum* race 1 strains, which were characterized as genetically homogenous based on four DNA-fingerprinting primers (BOX, ERIC, REP, and IS50). Their results, along with those obtained by LEE *et al.* (2012), led to the general consensus that BOX-PCR was the most sensitive method for the characterization of *P. s.* pv. *morsprunorum* strains. However, GILBERT *et al.* (2009) found BOX-PCR the least effective in assessing the genetic variability of *P. syringae* and *Pseudomonas viridiflava* strains. These authors thus adopted a combination of microbiological tests, rep-PCR (BOX, ERIC, and REP), and IS50-PCR for the visualization of *P. s.* pv. *syringae*, allowing them to confirm high genetic heterogeneity as well as existence of several isolate−host relationships. Therefore, use of several classical microbiological methods along with appropriate molecular

techniques is advised, as it would allow a more comprehensive and nuanced understanding of differences among tested strains.

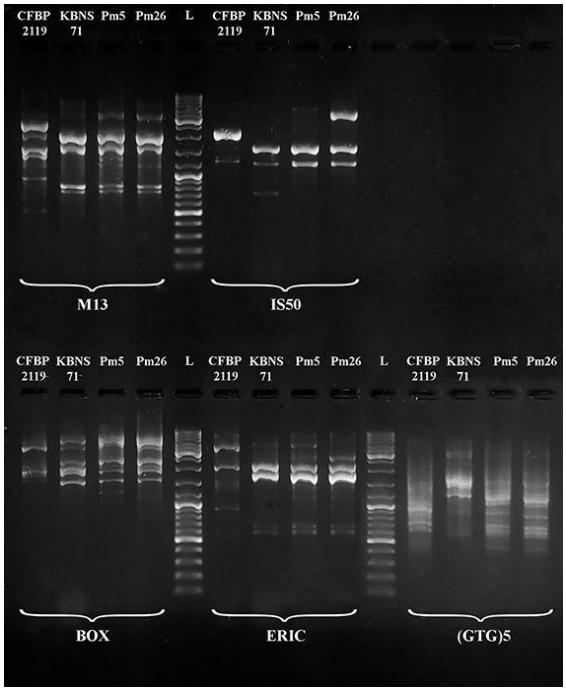

Figure 2. DNA fingerprinting patterns obtained with the BOX-, ERIC-, $(GTG)_5$-, M13-, and IS50-PCR for the two selected sweet cherry (Pm5) and plum (Pm26) *P. s.* pv. *morsprunorum* strains examined in the present study, one comparative (KBNS71) and one reference (CFBP 2119) *P. s.* pv. *morsprunorum* strain. The designation "L" refers to 1 kb GeneRuler DNA Ladder (Thermo Scientific).

In the present study, based on the constructed neighbor-joining phylogenetic trees with partial sequences of each of the four housekeeping genes (*gapA*, *gltA*, *gyrB*, and *rpoD*), the analyzed three Serbian sweet cherry and four Serbian plum strains were placed in the same tree cluster with the comparative Serbian strain KBNS71 and the additional four *P. s.* pv. *morsprunorum* race 1 strains sourced from the NCBI (CFBP 2116, CFBP 3840, R15244, and IO 25b), as shown in Figure 4. However, owing to their higher genetic similarity with strains of *P. s.* pvs. *avii* (CFBP 3846) and *persicae* (NCPPB 2254) compared to the *P. s.* pv. *morsprunorum* race 1 strains, *P. s.* pv. *morsprunorum* strains CFBP 6411 and IO 732 belonging to race 2 were separated in another tree cluster. In contrast to *P. s.* pv. *morsprunorum* races 1 and 2, *P. s.* pv. *syringae* strains RE3, RE05, and NCPPB 1652 were shown to be genetically heterogeneous. In

all generated trees, *P. azotoformans* P45A occupied a separate tree branch. Moreover, as can be seen from the neighbor-joining phylogenetic tree constructed with the concatenated sequences of all four genes presented in Figure 5, in line with the structure of trees based on partial sequences, all Serbian *P. s.* pv. *morsprunorum* strains (tested and comparative) were clustered together with four reference *P. s.* pv. *morsprunorum* race 1 strains from NCBI, signifying their genetic homogeneity. Strains belonging to race 1 were again clearly separated from *P. s.* pv. *morsprunorum* race 2 strains as well as strains of *P. cerasi* PL963 and *P. s.* pvs. *syringae*, *avii*, and *persicae*.

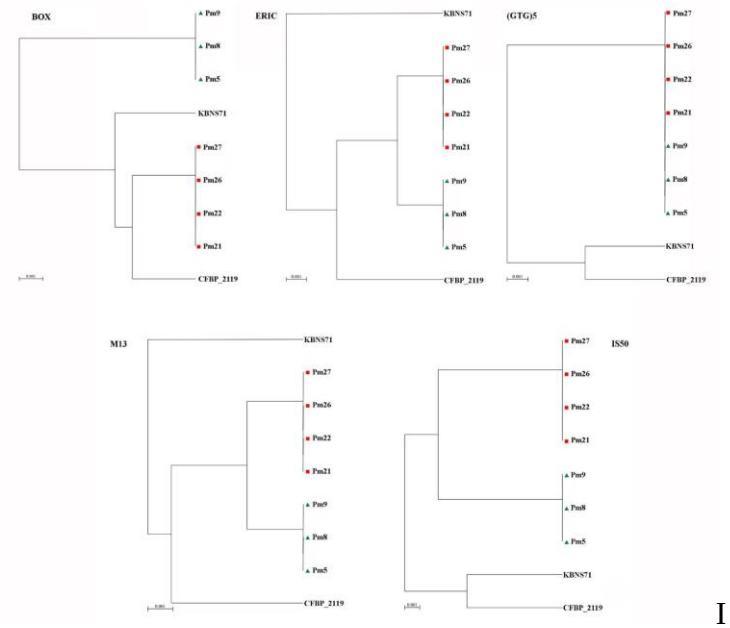

Figure 3. Dendrograms based on the UPGMA analysis of the BOX-, ERIC-, $(GTG)_5$-, M13-, and IS50-PCR patterns for three Serbian sweet cherry (denoted with green triangles) and four Serbian plum (labelled with red squares) *P. s.* pv. *morsprunorum* strains examined in the present study, one Serbian comparative sweet cherry strain (KBNS71) and one reference (CFBP 2119) *P. s.* pv. *morsprunorum* strain

HWANG *et al.* (2005) proposed that even the reduced MLST scheme—based on applying the methodology described by SARKAR and GUTTMAN (2004) to seven housekeeping genes (*acnB*, *cts*, *gapA*, *gyrB*, *pgi*, *pfk*, and *rpoD*) to retain just four genes (*gapA*, *gltA*, *gyrB*, and *rpoD*)—could enable appropriate typing of *P. syringae* strains, without affecting the

phylogenetic resolution. More recently, KALUZNA *et al*. (2010) successfully used the same four genes for distinguishing strains of *P. s.* pv. *morsprunorum* races 1 and 2, and *P. s.* pv. *syringae* isolated from sweet and sour cherry, plum, and hazelnut trees originating from Italy and Poland. In the present study, adoption of the strategy proposed by HWANG *et al*. (2005) to identify genes to be used in analyses enabled typing of the tested Serbian sweet cherry and plum strains and placing them in the same phylogenetic tree cluster together with different strains of *P. s.* pv. *morsprunorum* race 1 sourced from the NCBI. Therefore, the use of this restricted (four-gene-based) scheme could be sufficient for future typing studies of *P. s.* pv. *morsprunorum* and other species within the *P. syringae* species complex.

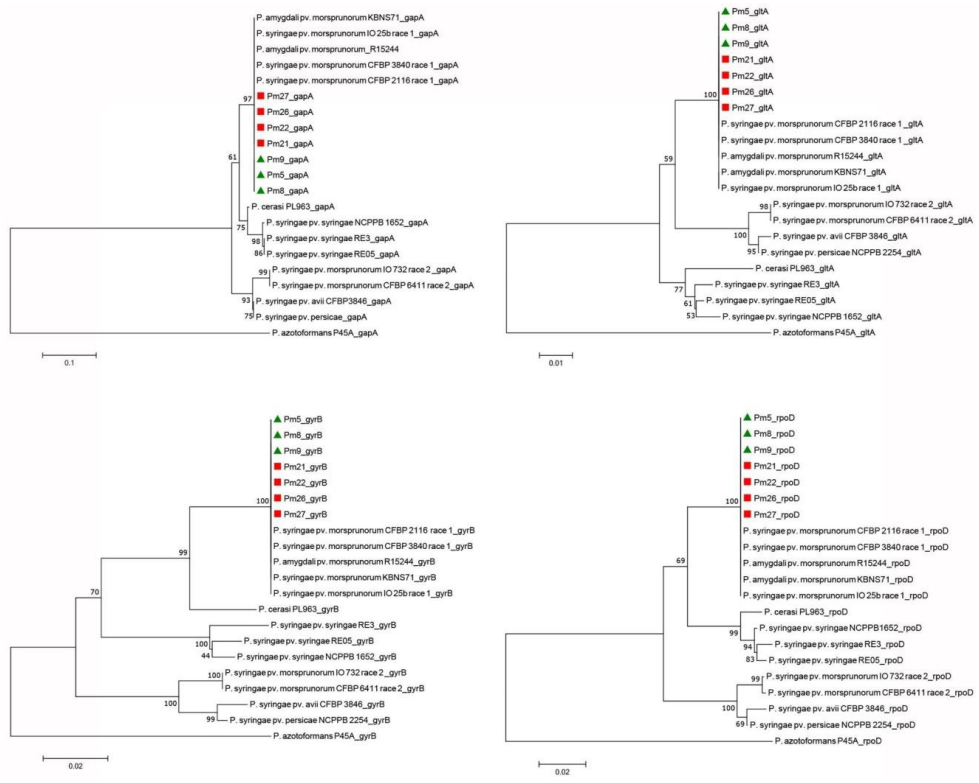

Figure 4. Neighbor-joining phylogenetic trees generated by utilizing the partial sequences of four sequenced genes (*gapA*, *gltA*, *gyrB*, and *rpoD*) for three Serbian sweet cherry (marked with triangles) and four Serbian plum (marked with rectangles) *P. s.* pv. *morsprunorum* strains, one comparative Serbian *P. s.* pv. *morsprunorum* strain KBNS71, six reference strains [CFBP 2116, CFBP 3840, CFBP 6411, R15244, IO 25b, and IO 732], and six additional strains of *P. cerasi and P. s.* pvs. *syringae*, *avii*, and *persicae* retrieved from the NCBI database. The tree was rooted with *P. azotoformans* strain P45A.

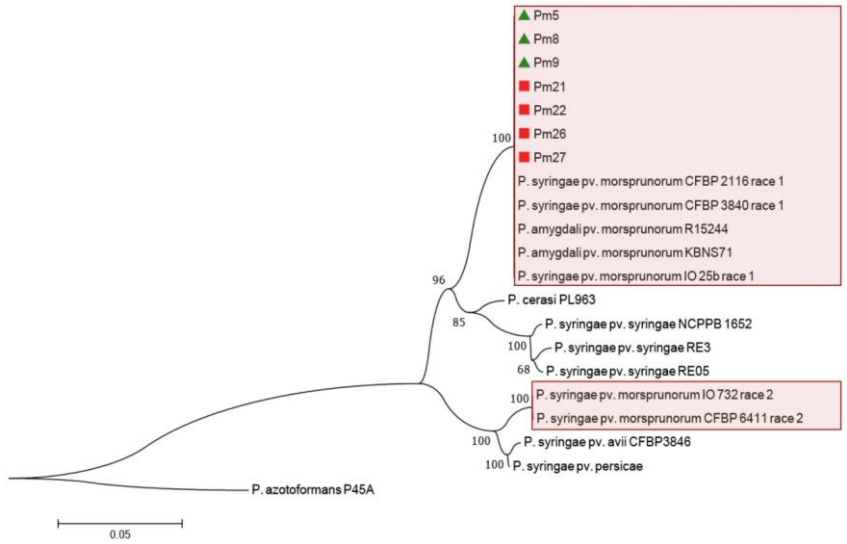

Figure 5. Neighbor-joining phylogenetic tree constructed with the concatenated sequences of four sequenced genes (*gapA*, *gltA*, *gyrB*, and *rpoD*) for three Serbian sweet cherry (marked with triangles) and four Serbian plum (marked with rectangles) *P. syringae* pv. *morsprunorum* strains, one comparative Serbian *P. s.* pv. *morsprunorum* strain KBNS71, six reference strains [CFBP 2116, CFBP 3840, CFBP 6411, R15244, IO 25b, and IO 732], and six additional strains of *P. cerasi* and *P. s.* pvs. *syringae*, *avii*, and *persicae* retrieved from the NCBI database. The tree was rooted with *P. azotoformans* strain P45A.

Phenotypic Characterization

All sweet cherry and plum strains examined in this study yielded positive pathogenicity results when tested on sweet and sour cherry fruitlets and bean pods. The first disease symptoms (darkening of tissue around the inoculation site) were observed on inoculated sweet and sour cherry fruitlets 48 hours after inoculation. All strains subsequently caused brownish, water-soaked superficial lesions, while pale grayish spots were observed on inoculated green bean pods. As shown in Table 3, the symptoms produced by the examined strains in the pathogenicity tests were the same as those caused by *P. s.* pv. *morsprunorum* race 1 reference CFBP 2119 and comparative strain KBNS71. On the other hand, in all experiments, fruitlets and pods treated with SDW were symptomless. Therefore, assays based on detached immature fruitlets and pods were shown to be reliable for the characterization of *P. syringae* strains originating from stone fruit, concurring with the observations made by KALUZNA and SOBICZEWSKI (2009), BALAŽ *et al.* (2016), HULIN *et al.* (2018), RUINELLI *et al.* (2019).

All tested strains from sweet cherry and plum were Gram negative, catalase positive, and HR positive, but were oxidase, pectolytic activity and arginine dihydrolase negative. They also produced fluorescent pigment on King B medium under UV light, and exhibited oxidative metabolism of glucose. These results correspond to fluorescent *Pseudomonas* Group Ia

(LELLIOTT *et al*., 1966). In GATTa tests, strains did not hydrolyze gelatin and were aesculin negative, while tyrosinase and L-tartrate were positive for all tested strains, and strains CFBP 2119 and KBNS71, corresponding to the *P. s.* pv. *morsprunorum* race 1 characteristics (LATORRE and JONES, 1979; SCHAAD *et al.*, 2001), as shown in Table 3. All strains, including reference and comparative strains, were sensitive to ten tested antibiotics. Thus, the phenotypic tests used in this study allowed the determination of sweet cherry and plum strains to the pathovar and race level.

Table 3. Summary of phenotypic characteristics of Pseudomonas strains from sweet cherry and plum

Isolate code	Host	Year of isolation	Locality	Pathogenicity characteristics	LOPAT	GATTa	Antibiogram
Pm5	Sweet cherry (*P. avium*)	2016	Topola, Serbia	*P. s.* pv. *morsprunorum* race 1	+ - - - +	- - + +	s
Pm8	Sweet cherry (*P. avium*)	2016	Topola, Serbia	*P. s.* pv. *morsprunorum* race 1	+ - - - +	- - + +	s
Pm9	Sweet cherry (*P. avium*)	2016	Topola, Serbia	*P. s.* pv. *morsprunorum* race 1	+ - - - +	- - + +	s
Pm21	Plum (*P. domestica*)	2020	Krušedol Selo, Serbia	*P. s.* pv. *morsprunorum* race 1	+ - - - +	- - + +	s
Pm22	Plum (*P. domestica*)	2020	Krušedol Selo, Serbia	*P. s.* pv. *morsprunorum* race 1	+ - - - +	- - + +	s
Pm26	Plum (*P. domestica*)	2020	Krušedol Selo, Serbia	*P. s.* pv. *morsprunorum* race 1	+ - - - +	- - + +	s
Pm27	Plum (*P. domestica*)	2020	Krušedol Selo, Serbia	*P. s.* pv. *morsprunorum* race 1	+ - - - +	- - + +	s
KBNS71	Sweet cherry (*P. avium*)	2012	Selenča, Serbia	*P. s.* pv. *morsprunorum* race 1	+ - - - +	- - + +	s
CFBP 2119	*Prunus cerasus*	1974	France	*P. s.* pv. *morsprunorum* race 1	+ - - - +	- - + +	s

In conclusion, the results obtained in the present study indicate existence of genetic diversity among *P. s.* pv. *morsprunorum* race 1. They further confirm that combining BOX-, ERIC-, M13-, and IS50-PCR analyses is a reliable method for assessing this diversity. Although MLSA findings indicated genetic homogeneity among all Serbian *P. s.* pv. *morsprunorum* strains (tested and comparative), this method is nonetheless valuable for species, pathovar, and race separation. Phenotypic features were uniform in case of *P. s.* pv. *morsprunorum* sweet cherry and plum strains.

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REFERENCES

BALAŽ, J., R., ILIČIĆ, V., OGNJANOV, Ž., IVANOVIĆ, T., POPOVIĆ (2016): Etiology of bacterial canker on young sweet cherry trees in Serbia. Journal of Plant Pathology, *98*(2): 285-294.

BERGE, O., C.L., MONTEIL, C., BARTOLI, C., CHANDEYSSON, C., GUILBAUD, D.C., SANDS, C.E., MORRIS (2014): A user's guide to a data base of the diversity of *Pseudomonas syringae* and its application to classifying strains in this phylogenetic complex. PloS one, *9*(9): e105547.

BERESWILL, S., P., BUGERT, B., VOLKSCH, M., ULLRICH, C., BENDER, K., GEIDER (1994): Identification and relatedness of coronatine-producing *Pseudomonas syringae* pathovars by PCR analysis and sequence determination of the amplification products. Applied and Environmental Microbiology, *8*: 2924-2930.

BULTREYS, A., M., KALUZNA (2010): Bacterial cankers caused by *Pseudomonas syringae* on stone fruit species with special emphasis on the pathovars *syringae* and *morsprunorum* race 1 and race 2. Journal of Plant Pathology, *92*(1, Supplement): S21-S33.

GILBERT, V., F., LEGROS, H., MARAITE, A. BULTREYS (2009): Genetic analyses of *Pseudomonas syringae* isolates from Belgian fruit orchards reveal genetic variability and isolate-host relationships within the pathovar syringae, and help identify both races of the pathovar morsprunorum. European Journal of Plant Pathology, *124*(2): 199-218.

GIOVANARDI, D., FERRANTE, P., SCORTICHINI, M., E. STEFANI (2018): Characterisation of *Pseudomonas syringae* isolates from apricot orchards in north-eastern Italy. European Journal of Plant Pathology, *151*(4): 901-917.

ERCOLANI, G.L., HAGEDORN, D.J., KELMAN, A., R.E., RAND (1974): Epiphytic survival of *Pseudomonas syringae* on hairy vetch in relation to epidemiology of bacterial brown spot of bean in Wisconsin. Phytopathology, *64*(10): 1330-1339.

FELSENSTEIN, J. (2004): PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

FREIGOUN, S.O., J.E., CROSSE (1975): Host relations and distribution of a physiological and pathological variant of *Pseudomonas morsprunorum*. Annals of Applied Biology, *81*(3): 317-330.

GARDAN, L., SHAFIK, H., BELOUIN, S., BROCH, R., GRIMONT, F., P.A.D., GRIMONT (1999): DNA relatedness among the pathovars of *Pseudomonas syringae* and description of *Pseudomonas tremae* sp. nov. and *Pseudomonas cannabina* sp. nov. (ex Sutic and Dowson 1959). International Journal of Systematic and Evolutionary Microbiology, *49*(2): 469-478.

GAVRILOVIĆ, V., Ž., IVANOVIĆ, T., POPOVIĆ, S., ŽIVKOVIĆ, S., STANKOVIĆ, T., BERIĆ, Đ., FIRA (2013): Genetic characterization of pathogenic fluorescent pseudomonads isolated from necrotic cherry and plum buds in Serbia. Genetika, *45*(3): 953-961.

GILBERT, V., F., LEGROS, H., MARAITE, A., BULTREYS (2009): Genetic analysis of *Pseudomonas syringae* from Belgian fruit orchards reveal genetic variability and isolate-host relationships within the pathovar *syringae*, and help identify both races of the pathovar *morsprunorum*. European Journal of Plant Pathology, *124*: 199-218.

HULIN, M., T., MANSFIELD, J. W., BRAIN, P., XU, X., JACKSON, R. W., R. J. HARRISON (2018): Characterization of the pathogenicity of strains of *Pseudomonas syringae* towards cherry and plum. Plant pathology, *67*(5): 1177-1193.

HWANG, M.S., R.L., MORGAN, S.F., SARKAR, P.W., WANG, D.S., GUTTMAN (2005): Phylogenetic characterization of virulence and resistance phenotypes of *Pseudomonas syringae*. Applied and Environmental Microbiology, *71*(9): 5182-5191.

ILIČIĆ, R. (2016): Bacterial dieback of sweet cherry (*Prunus avium* L.). PhD Thesis, University of Novi Sad, Faculty of Agriculture, SRB, *187*.

ILIČIĆ, R., A., JELUŠIĆ, S., MARKOVIĆ, G., BARAĆ, F., BAGI, T., POPOVIĆ (2022): *Pseudomonas cerasi*, the new wild cherry pathogen in Serbia and the potential use of recG helicase in bacterial identification. Annals of Applied Biology, *180* (1): 140-150.

ILIČIĆ, R., J., BALAŽ, V., OGNJANOV, D., JOŠIĆ, S., VLAJIĆ, M., LJUBOJEVIĆ, T., POPOVIĆ (2018): Evaluation of cherry cultivar susceptibility to bacterial canker and leaf spot disease. Journal of Phytopathology, *166*: 799–808.

ILIČIĆ, R., J., BALAŽ, V., OGNJANOV, T., POPOVIĆ (2021a): Epidemiology studies of *Pseudomonas syringae* pathovars associated with bacterial canker on sweet cherry in Serbia. Plant Protection Science, *57*(3), 196–205.

ILIČIĆ, R., T., POPOVIĆ, S., MARKOVIĆ, A., JELUŠIĆ, F., BAGI, S., VLAJIĆ, S., STANKOVIĆ (2021b): Genetic diversity of *Pseudomonas syringae* pv. *syringae* isolated from sweet cherry in southern and northern regions in Serbia. Genetika, *53*(1): 247-262.

ILIČIĆ, R., T., POPOVIĆ, S., VLAJIĆ, V., OGNJANOV (2019): Foliar pathogens of sweet and sour cherry in Serbia. Acta Agriculturae Serbica, *48*: 107-118.

KAŁUŻNA, M., P., ALBUQUERQUE, F., TAVARES, P., SOBICZEWSKI, J., PUŁAWSKA (2016): Development of SCAR markers for rapid and specific detection of *Pseudomonas syringae* pv. *morsprunorum* races 1 and 2, using conventional and real-time PCR. Applied Microbiology and Biotechnology, *100*(8): 3693-3711.

KALUZNA, M., P., FERRANTE, P., SOBICZEWSKI, M. SCORTICHINI (2010): Characterization and genetic diversity of *Pseudomonas syringae* from stone fruits and hazelnut using repetitive-PCR and MLST. Journal of Plant Pathology, *92*(3): 781-787.

KAŁUŻNA, M., P., SOBICZEWSKI (2009): Virulence of *Pseudomonas syringae* pathovars and races originating from stone fruit trees. Phytopathologia, *54*: 71-78.

KHEZRI, M., M., MOHAMMADI (2018): Identification and characterization of *Pseudomonas syringae* pv. *syringae* strains from various plants and geographical regions. Journal of Plant Protection Research, *58*(4): 354–361.

KIMURA, M. (1980): A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, *16*: 111–120.

KUMAR, S., G., STECHER, M., LI, C., KNYAZ, K., TAMURA (2018): MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution, *35*(6): 1547.

LATORRE, B.A., A.L., JONES (1979): *Pseudomonas morsprunorum*, the cause of bacterial canker of sour cherry in Michigan and its epiphytic association with *P. syringae*. Phytopathology, *69*: 335-339.

LE MARREC, C., B., HYRONIMUS, P., BRESSOLLIER, B., VERNEUIL, M.C., URDACI (2000): Biochemical and genetic characterization of coagulin, a new antilisterial bacteriocin in the pediocin family of bacteriocins, produced by *Bacillus coagulans* I4. Applied and Environmental Microbiology, *66*(12): 5213-5220.

LEE, Y.S., H.S., KOH, S.H., SOHN, Y.J., KOH, J.S., JUNG (2012): Genetic diversity among *Pseudomonas syringae* pv. *morsprunorum* isolates from *Prunus mume* in Korea and Japan by comparative sequence analysis of 16S rRNA gene. The Plant Pathology Journal, *28*(3): 295-298.

LELLIOTT, R.A., E., BILLING, A.C., HAYWARD (1966): A determinative scheme for the fluorescent plant pathogenic Pseudomonads. Journal of Applied Bacteriology, *29*(3): 470-489.

LOUWS, F.J., D.W., FULBRIGHT, C.T., STEPHENS, F.J., DE BRUIJN (1994): Specific genomic fingerprints of phytopathogenic *Xanthomonas* and *Pseudomonas* pathovars and strains generated with repetitive sequences and PCR. Applied and Environmental Microbiology, *60*(7): 2286-2295.

MARTIN-SANZ, A., M.P., DE LA VEGA, J., MURILLO, C., CAMINERO (2013): Strains of *Pseudomonas syringae* pv. *syringae* from pea are phylogenetically and pathogenically diverse. Phytopathology, *103*(7): 673-681.

NOSRATNEZHAD, F., K., ROUHRAZI, N., KHEZRINEZHAD (2018): Characterization and genetic diversity of *Pseudomonas syringae* isolates from stone fruits in north-western Iran. Journal of Phytopathology, *166*:516–524.

PARKINSON, N., R., BRYANT, J., BEW, J., ELPHINSTONE (2011): Rapid phylogenetic identification of members of the *Pseudomonas syringae* species complex using the *rpo*D locus. Plant Pathology, *60*(2): 338-344.

PERRIERE, G., M., GOUY (1996): WWW-query: an on-line retrieval system for biological sequence banks. Biochimie, *78*(5): 364-369.

POPOVIĆ, T., A., JELUŠIĆ, I., DIMKIĆ, S., STANKOVIĆ, D., POŠTIĆ, G., ALEKSIĆ, S., VELJOVIĆ JOVANOVIĆ (2019): Molecular characterization of *Pseudomonas syringae* pv. *coriandricola* and biochemical changes attributable to the pathological response on its hosts carrot, parsley, and parsnip. Plant Disease, *103*(12): 3072-3082.

RADEMAKER, J.L., B., HOSTE, F.J., LOUWS, K., KERSTERS, J., SWINGS, L., VAUTERIN, F.J., DE BRUIJN (2000): Comparison of AFLP and rep-PCR genomic fingerprinting with DNA-DNA homology studies: *Xanthomonas* as a model system. International Journal of Systematic and Evolutionary Microbiology, *50*(2): 665-677.

RUINELLI, M., J., BLOM, T.H., SMITS, J.F., POTHIER (2019): Comparative genomics and pathogenicity potential of members of the *Pseudomonas syringae* species complex on *Prunus* spp. BMC genomics, *20*(1): 1-16.

SARKAR, S.F., D.S., GUTTMAN (2004): Evolution of the core genome of *Pseudomonas syringae*, a highly clonal, endemic plant pathogen. Applied and Environmental Microbiology, *70*(4): 1999-2012.

SCHAAD, N.W., J.B., JONES, W., CHUN (2001): Laboratory Guide for Identification of Plant Pathogenic Bacteria. The American Phytopathological Society, St. Paul, MN., USA.

SCORTICHINI, M., M., BIOCCA, M.P., ROSSI (1995): *Pseudomonas syringae* pv. *morsprunorum* on wild cherry for timber production: outbreak and field susceptibility. Forest Pathology, *25*(6-7): 343-350.

VERSALOVIC, J., M., SCHNEIDER, F. J., DE BRUIJN, J.R., LUPSKI (1994): Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. Methods in Molecular and Cellular Biology, *5*(1): 25-40.

VICENTE, J., S., ROBERTS (2007): Discrimination of *Pseudomonas syringae* isolates from sweet and wild cherry using rep-PCR. European Journal of Plant Pathology, *117*:383-392.

VICENTE, J.G., J.P., ALVES, K., RUSSELL, S.J., ROBERTS (2004): Identification and discrimination of *Pseudomonas syringae* isolates from wild cherry in England. European Journal of Plant Pathology, *110*(4): 337-351.

WEINGART, H., B., VÖLKSCH (1997): Genetic fingerprinting of *Pseudomonas syringae* pathovars using ERIC- , REP- , and IS50- PCR. Journal of Phytopathology, *145*(8- 9): 339-345.

KARAKTERIZACIJA *Pseudomonas syringae* PV. *morsprunorum* POREKLOM SA TREŠNJE I ŠLJIVE U SRBIJI

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Izvod

Sojevi fitopatogene bakterije iz roda *Pseudomonas* poreklom iz simptomatskih (bakteriozna pegavost) uzoraka listova trešnje (Topola, Šumadija) i šljive (Krušedol selo, Srem) izolovani su tokom 2016. i 2020. godine. Na osnovu klasičnih mikrobioloških metoda, LOPAT (+---+), GATTa (--++) i testova patogenosti na odvojenim plodovima trešnje i višnje, i mahunama boranije, svi sojevi su identifikovani kao *P. syringae* pv. *morsprunorum*. Detekcija *cfl* gena utvrdila je da sojevi pripadaju rasi 1. Na osnovu DNA profila dobijenih sa četiri rep- (BOX i ERIC), RAPD- (M13) i IS50-PCR (IS50) metode, utvrđeno je da su sojevi *P s.* pv. *morsprunorum* sa trešnje i šljive (sedam testiranih), uporedni KBNS71 i referentni soj CFBP 2119 genetski heterogeni. Nasuprot tome, MLSA je primenom šeme zasnovane na četiri gena (g*apA, gltA, gyrB* i *rpoD*) ukazala na genetsku homogenost svih testiranih sojeva sa trešnje i šljive zajedno sa *P. s.* pv. *morsprunorum* rase 1 sojevima iz NCBI. Iako su sojevi sa trešnje i šljive korišćeni u ovoj studiji bili 100% identični u MLSA, oni se mogu razlikovati po genima virulentnosti, stoga bi sekvencioniranje genoma moglo koristiti u cilju određivanja podgrupa sojeva na osnovu domaćina.

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