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Original scientific paper

Characterization of *Pectobacterium carotovorum* subsp. *carotovorum* isolates from a recent outbreak on cabbage in Bosnia and Herzegovina

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SUMMARY

The causal agent of soft rot disease associated with a cabbage outbreak in Semberija region, Bosnia and Herzegovina, in 2018 was identified and characterized. Symptoms appeared in the form of water-soaked lesions on leaves and specific odour. Disease incidence ranged from 20% to 30%. The causal pathogen was isolated on nutrient agar (NA), King's B and crystal violet pectate (CVP) media. Eight creamy-white, round and convex bacterial isolates, which produced characteristic pits on CVP medium were taken as representative. They were gram negative, facultative anaerobe, oxidase negative, catalase positive, nonfluorescent on King's B medium, levan and arginine dehydrolase negative. The isolates were able to cause soft rot on cabbage and potato tuber slices 24 h after inoculation under conditions of high relative humidity. Polymerase chain reaction (PCR) was performed for preliminary identification by using three specific primer sets: F0145/E2477 (specific for *Pectobacterium carotovorum* subsp. *carotovorum*), Br1f/L1r (specific for *P. carotovorum* subsp. *brasiliensis*) and ECA1f/ECA2r (specific for *P. atrosepticum*). All isolates produced the band size of 666 bp with F0145/E2477 primer pair, indicating that they belong to the species *P. carotovorum* subsp. *carotovorum*. Further genetic characterization was based on sequence analysis of the *gapA* and *mdh* housekeeping genes. BLAST analysis confirmed 99.39% (Q. cover 100%, E. value 0.0) and 100% (Q. cover 100%, E. value 0.0) identity of the isolates with *P. carotovorum* subsp. *carotovorum* strains deposited in the NCBI database as M34 (KY047594) for *gapA* and Pcc t0437 (KC337296) for *mdh* genes, respectively. Phylogenetic analysis showed genetic homogeneity among the cabbage isolates.

Keywords: *Pectobacterium*; cabbage; identification; characterization

INTRODUCTION

Cabbage (*Brassica oleracea* L. var. *capitata* L.; *Brassicaceae*) is one of the most widely cultivated cruciferous worldwide (Chiang et al., 1993). It is currently grown in more than 90 countries on five continents (Chiang et al., 1993). The best conditions for cabbage production exist in regions with humid and cool climate (Chiang et al., 1993; Bewick, 1994). Based on data in the Food and Agriculture Organization (FAO) statistical database, world production of cabbage and other brassicas was 71.451.138 metric tons in 2017 (http://www.fao.org/faostat/). Cabbage is a productive vegetable with yields of 50-60 tons per hectare (Watanabe & Pehu, 1997). In the Statistical Yearbook of the Republic of Serbia for 2018, cabbage and kale were presented together as an important vegetable species with annual production rate of 24.9 t/ha (https://www.stat.gov.rs).

Cabbage diseases reduce yields, and bacterial soft rot is one of the most destructive bacterial diseases, causing the highest overall production loss (Agrios, 2006). The disease is present on a wide range of plant species worldwide, including several economically important plant species. Yield loss differs from one country to another, depending on climate and growing conditions (Perombelon & Kelman, 1980). Soft rot occurs on crops in the field, especially during the heading period, during transport or in storage (Anonimous, 1990). Post-harvest losses caused by bacterial soft rot have been estimated at 15-30% (Agrios, 2006). Latently infected vegetables (no visible symptoms) kept under inadequate storage conditions (temperature from 30-35 °C and relatively high humidity) may be lost in total (Bhat et al., 2010). Soft rot symptoms are similar on most hosts and can be easily recognized as soft, wet, cream-coloured decompressed tissue often surrounded by dark margins. A characteristic odour occurs with disease progress.

Bacterial species mainly belonging to the genus *Pectobacterium* (formerly *Erwinia*) are responsible for soft rot disease, including several species described in the past and more recently: *P. carotovorum* (*P. c.* pv. *carotovorum*, *P. c.* subsp. *brasiliense*, *P. c.* subsp. *odoriferum*), *P. atrosepticum*, *P. betavasculorum, P. wasabiae, P. cacticida*, *P. parmentieri, P. aroidearum, P. polaris*, *P. peruviense*, *P. punjabense*, *P. aquaticum*, *P. zantedeschiae* and *Candidatus* P. maceratum (Gardan et al., 2003; Baghaee-Ravari et al., 2011; Nabhan et al., 2012a, 2013; Czajkowski et al., 2015; Khayi et al., 2015, 2016; Dees et al., 2017; Waleron et al., 2018, 2019; Sarfraz et al., 2018; Shirshikov et al., 2018; Li et al., 2018, 2019; Zaczek-Moczydłowska et al., 2019). The species *P. aroidearum*, *P. peruviense*, *P. polaris* and *Candidatus* P. maceratum, previously classified as *P. carotovorum,* have been separated and proposed as four new species (Li et al. 2018). The species *P. zantedeschiae* was previously assigned to *P. atrosepticum* (Popović et al., 2017; Waleron et al., 2019). *Pectobacterium* species are listed in the top ten bacterial plant pathogens with economic impact (Mansfield et al., 2012). The plant pathogenic bacterium *P. c.* subsp. *carotovorum* is the most common pathogen causing soft rot and affecting plants of at least 16 dicotyledonous and 11 monocotyledonous angiosperm families, and it has been studied most extensively (Ma et al., 2007; Yishay et al., 2008). As the causal agent of cabbage soft rot it has been found in Jordan (Rajeh & Hamed, 2000), Malaysia (Nazerian et al., 2011), India (Bhat et al., 2010) and more recently detected in Iran (Rafiei et al., 2015), Turkey (Aksoy et al., 2017) and Poland (Oskiera et al., 2017). Soft rot shows no visible symptoms on harvested vegetables (latent infection) but disease progress goes on during post-harvest stages, especially under high temperature and humidity, making the problem more devastating (Bhat et al., 2012).

Taxonomic affiliation based on *multilocus sequence analysis* (MLSA) is used nowadays for inferring accurate phylogeny and providing strong support in identification of bacterial species and genera (Popović et al., 2019a, 2019b; Li et al., 2019; Tambong, 2019). The housekeeping genes that are commonly used to differentiate isolates of *Pectobacterium* species are: *acnA*, *gapA*, *mdh*, *pgi*, *mtlD*, *proA*, *rpoS*, *recA* and *dnaX* (Zeigler, 2003; Ma et al., 2007; Nabhan et al., 2012a).

In Serbia, soft rot of cabbage was noticed in Bačka region during the 1990s, and *Erwinia carotovora subsp. carotovora* was identified as its causal agent (Arsenijević & Obradović, 1996; Mitrović, 1997). After that, there were no new records of disease outbreaks in that Serbian region until 2016 when *P. carotovorum* was identified as the causal agent of cabbage soft rot in different locations in Serbia (Vlajić et al., 2017).

During 2018, bacterial soft rot symptoms were observed on cabbage in Semberija, a region in the Republic of Srpska (Bosnia and Herzegovina) bordering on Serbia, and symptoms included water-soaked lesions on leaves and the characteristic odour. Disease incidence ranged between 20% and 30%. Therefore, the objective of this research was to identify and characterize the causal agent of soft rot disease associated with the observed cabbage disease outbreak.

MATERIALS AND METHODS

Pathogen isolation

Symptoms of soft rot (Figure 1) were observed in Semberija in July 2018 in the form of water-soaked lesions surrounded by dark margins on leaves. The specific odour was also noticed.

Samples of symptomatic soft rot cabbage heads were collected (Figure 2) and the causal pathogen isolated and identified. Cabbage leaves were first washed under tap water, and then dried at room temperature. Small leaf pieces sampled from margins between healthy and diseased tissue were homogenized in sterile distilled water (SDW). Isolation was performed on nutrient agar (NA), King's B and crystal violet pectate (CVP) media by plating the obtained suspension.

After a 48 h incubation period at the temperature of 26 °C, many bacterial colonies were formed. A total of eight isolates (coded as: Pcc1, Pcc3, Pcc5, Pcc8, Pcc10, Pcc13, Pcc14, Pcc16) producing characteristic pits on CVP were taken as representatives. The isolates were purified on NA medium and kept as pure cultures at the temperature of -20 **°C** in Luria Bertani broth (LB) supplemented with 20% of sterile glycerol.

Figure 1. Symptoms of soft rot on cabbage (photo Lj. Vuković)

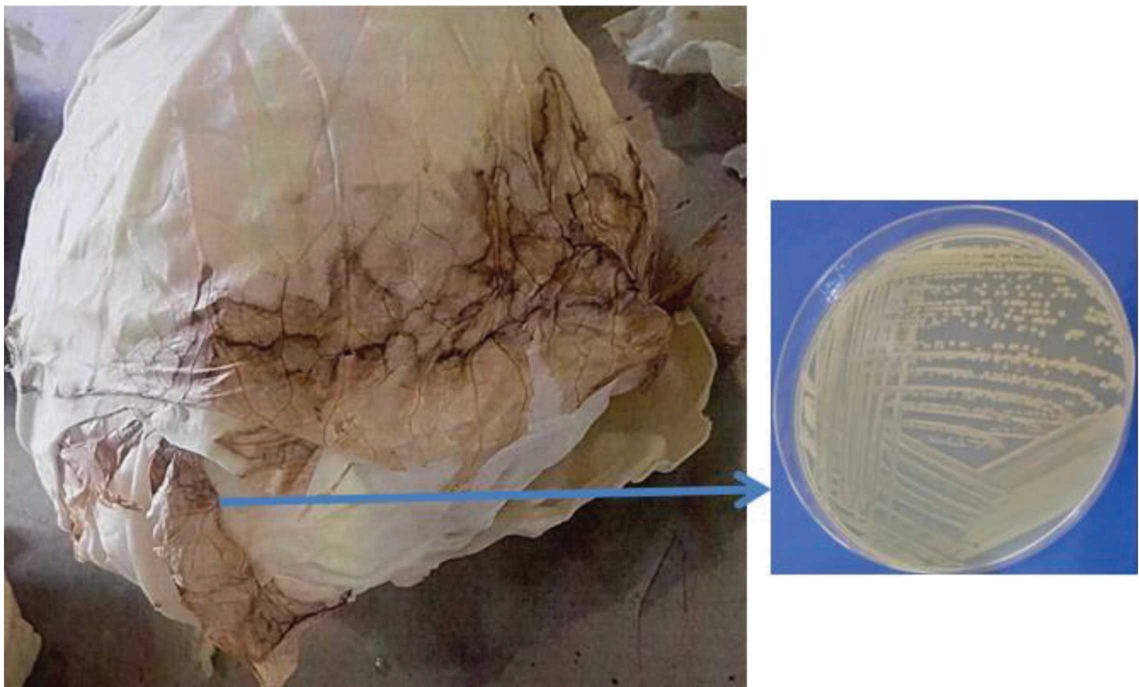

Figure 2. A symptomatic soft rot cabbage head (left), bacterial colonies of pure culture on NA medium (photo T. Popović)

Phenotypic characterization

All isolates were initially assayed by following bacteriological tests: Gram reaction, fluorescence on King's B medium, oxidative/fermentative metabolism of glucose (O/F test), oxidase and catalase test and presence of arginine dehydrolase (Schaad et al., 2001).

The ability of the test isolates to macerate potato tubers was determined using a pectolytic test on potato tuber slices. Healthy potato tubers with no visible damage were washed, disinfected with 95% ethanol and cut into slices of 1-2 cm thickness. Holes of 0.7 cm diameter were made at the center of each potato slice. The slices were placed in Petri dishes on wet filter papers and inoculated by filling the holes with bacterial suspension (c. 10^7 CFU mL^{-1}). SDW served as a negative control treatment. The inoculated slices were kept under high relative humidity at room temperature (22 ± 1 °C) for 48 h. The test was performed in three replicates per isolate.

Pathogenicity was tested on cabbage slices inoculated with bacterial suspensions (10^7-10^8 CFU mL^{-1}). The inoculated slices were incubated at room temperature and high relative humidity in a moist chamber. Cabbage slices treated with SDW served as the negative control. The experiment was conducted in four replications. Four cabbage slices were inoculated per isolate and control. After 24 h, the appearance of rotten tissue was monitored. Reisolation from the rotten cabbage slices was performed on NA using the same procedure as for pathogen isolation.

Polymerase chain reaction (PCR)

Genomic DNA extraction was performed using the heat treatment method. A loopfull of pure bacterial culture of each isolate, grown on NA for 48 h at 26 °C, was suspended in 500 µl of SDW and adjusted to concentration of 10^8 CFU mL^{-1}. The suspensions were homogenized using vortex, heated at 95 °C for 10 min and shortly cooled on ice.

PCR procedures were conducted using three specific primer sets for fast determination of soft rot-causing *Pectobacterium* species previously confirmed in Serbia, i.e. F0145/E2477 for *P. carotovorum* subsp. *carotovorum* (Kettani-Halabi et al., 2013), Br1f/L1r for *P. carotovorum* subsp. *brasiliensis* (Duarte et al., 2004) and ECA1f/ECA2r for *P. atrosepticum* (De Boer & Ward, 1995) (Table 1). The PCR was carried out in a final reaction volume of 25 µl, which contained 12.5 µL of DreamTaq Green PCR Master Mix (Thermo Fisher Scientific), 1 µL of template DNA, 1 µL of each primer used (10 µM) and 9.5 µL of ultrapure DNase/RNase-free water (Gibco, UK). PCR reactions using the primers F0145/E2477 were carried out according to the following conditions: initial denaturation was at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 2 min, and a final extension step at 72 °C for 10 min; the thermal regime for the primers BR1f/L1r was: initial denaturation at 94 °C for 2 min, 25 cycles of denaturation at 94 °C for 45 sec, annealing at 62 °C for 45 sec, extension at 72 °C for 90 sec followed by a terminal extension step at 72 °C for 10 min, while conditions for the ECA1f/ECA2r primer were: initial denaturation at 95 °C for 5 min, 40 cycles of denaturation at 94 °C for 30 sec, annealing at 62 °C for 45 sec, extension at 72 °C for 45 sec and final extension at 72 °C for 8 min. After amplification, PCR products were checked for the presence of bands on the expected positions by electrophoresis on 1.5% agarose gel stained with ethidium bromide (0.5 µg ml^{1}).

Table 1. Primers used in the study

Primer name	Primer sequence	Reference	Fragment length (bp)
F0145	5′-TACCCTGCAGATGAAATTATTGATTGTTGAAGAC-3′	Kettani-Halabi et al. (2013)	666
E2477	5′-TACCAAGCTTTGGTTGTTCCCCTTTGGTCA-3′		
ECA1f	5′-CGGCATCATAAAAACACG-3′	De Boer & Ward (1995)	690
ECA2r	5′-GCACACTTCATCCAGCGA-3′		
Br1f	5′-GCGTGCCGGGTTTATGACCT-3′	Duarte et al. (2004)	322
L1r	5′-CAAGGCATCCACCGT-3′		
gapA326F	5′-ATCTTCCTGACCGACGAAACTGC-3′	Ma et al. (2007)	450
gapA845R	5′-ACGTCATCTTCGGTGTAACCCAG-3′		
mdh2	5′-GCGCGTAAGCCGGGTATGGA-3′	Moleleki et al. (2013)	500
mdh4	5′-CGCGGCAGCCTGGCCCATAG-3′		

Multilocus sequence analysis *(MLSA)*

Total genomic DNA extraction was performed by using a modified CTAB method (Ausubel et al., 2003). The obtained DNA was dissolved in 100 µL of TE buffer and kept at -20 °C.

The relatedness among the isolates was assessed by nucleotide sequencing of two housekeeping genes, including glyceraldehydes-3-phosphate dehydrogenase (*gapA*) and malate dehydrogenase (*mdh*) (Table 1). PCR reactions were performed in a total reaction volume of 25 µL, consisting of 12.5 µL Master Mix (Thermo Fisher Scientific), 9.5 µL of ultrapure DNase/RNase-free water (Gibco, UK),1 µL of DNA and 1 µL of each of the primers (10 µM). The PCR conditions were set as follows: initial denaturation at 95 °C for 5 min, followed by 35 amplification cycles of denaturation at 94 °C for 30 sec, annealing at 52 °C for 30 sec, extension at 72 °C for 60 sec, and final extension phase at 72 °C for 7 min (Onkendi & Moleleki, 2014). Amplified products were visualized by electrophoresis on a 1.5% agarose gel stained with ethidium bromide (0.5 µg/ml), under UV light. The PCR products were purified using the QIAquick/250 Gel Extraction and Purification Kits (QIAGEN GmbH, Hilden, Germany) and sequenced in Macrogen sequencing service (Amsterdam, Netherlands).

The obtained sequences were identified using the nucleotide BLAST (Basic Local Alignment Search Tool) search tool based on data from the NCBI (National Center for Biotechnology Information). DNA sequences were manually edited and aligned using ClustalW tool in BioEdit (ver. 7.0.5). Considering the homogeneity of tested cabbage isolates, the sequences of two isolates, Pcc1 and Pcc10, were deposited to GenBank under accession numbers: MT188695, MT188696 for *gapA* gene and MT188697, MT188698 for *mdh* gene, respectively. Sequences of different *Pectobacterium* spp. retrieved from the NCBI database were used for comparison (Table 2).

For the MLSA, sequences were trimmed to the sizes of 384 nt for *gapA* and 280 nt for the *mdh* gene. Neighbor-Joining (NJ) phylogenetic trees were constructed in Mega 7 (Tamura et al., 2011) software, using the Kimura two-parameter distance model. The *Yersinia pestis* strain Yp91001 (acc. nos. EF550686 for *gapA* and EF550791 for *mdh*) was used as an out group.

RESULTS AND DISCUSSION

Isolation of the causal agent of soft rot disease on cabbage resulted in the growth of numerous bacterial colonies, and dominating were those that formed piths

Table 2. Comparative strains of *Pectobacterium* spp. retrieved from NCBI database

Bacterium	Strain code	Country	Host	Accession number	
				gapA	*mdh*
Pectobacterium carotovorum subsp. *carotovorum*	M34	France	*Solanum tuberosum*	KY047594	-
	Pcct0437	Finland	*Solanum tuberosum*	-	KC337296
	Ecc63	Netherlands	*Solanum tuberosum*	EF550675	EF550781
	Ecc71	Netherlands	*Solanum tuberosum*	EF550674	EF550780
	Ecc21	Netherlands	*Solanum tuberosum*	EF550673	EF550779
	JKI4.3.8	Germany	*Brassica oleracea*	HM156849	HM156972
	M30	Syria	*Solanum tuberosum*	HM156844	HM156967
	A18	Syria	*Solanum tuberosum*	HM156825	HM156947
	C144	Syria	*Solanum tuberosum*	HM156835	HM156957
	C267	Syria	*Solanum tuberosum*	HM156836	HM156959
	C137	Syria	*Solanum tuberosum*	HM156829	HM156951
Pectobacterium parmentieri	WPP163	USA	*Solanum tuberosum*	NC013421	NC 013421
Pectobacterium wasabiae	SCRI488	Japan	*Eutrema wasabi*	EF550680	EF550785
Pectobacterium atrosepticum	36A	Belarus	*Solanum tuberosum*	CP024956	CP024956
Pectobacterium betavasculorum	ATCC43762	USA	*Beta vulgaris*	FJ895843	FJ895845

on CVP medium. On NA, colonies of pure culture were creamy-white, round and convex. All isolates were gram negative, facultative anaerobe, oxidase negative, catalase positive, non-fluorescent on King's B medium, negative for levan formation and activity of arginine dehydrolase, and positive for pectolytic activity on potato tuber slices after 24 h (Table 3).

Inoculated cabbage slices developed disease symptoms 24 h after inoculation. Negative controls remained healthy. Reisolates were confirmed to be identical to the original ones using the F0145/E2477 primer pair.

Soft rot disease may be caused by many bacteria of the genera *Bacillus*, *Pseudomonas* or *Pectobacterium* (Agrios, 2006). Isolates originating from soft rotted cabbage have been determined preliminarily to belong to the genus *Pectobacterium* based on results obtained by phenotypic characterization (Mauzey et al., 2011; Oskiera et al., 2017). Pectolytic activity on potato slices is a trait that can differentiate bacteria of the genus *Pectobacterium* from some of *Pseudomonas* spp., such as *Pseudomonas cannabina* pv. *alisalensis,* which cannot cause maceration of potato tuber slices (Mauzey et al., 2011).

Preliminary identification based on the use of *Pectobacterium* specific primers showed that all cabbage isolates produced 666 bp band using the F0145/E2477 primer pair, indicating the presence of *P. carotovorum* subsp. *carotovorum*. PCR reactions using the Br1f/L1r and ECA1f/ECA2r primers were negative.

The NCBI nucleotide BLAST analysis of *gapA* and *mdh* housekeeping genes showed the highest similarity of tested isolates with *P. carotovorum* subsp. *carotovorum* strains M34 (99.39%) and Pcct0437 (100%), respectively, both isolated from potato in France (M34) and Finland (Pcct0437).

The obtained NJ phylogenetic tree, made based on the *gapA* partial nucleotide sequences (Figure 3), separated eight tested cabbage isolates and ten comparative *P. carotovorum* subsp. *carotovorum* strains from the NCBI in two clusters (cluster I and cluster II). All cabbage isolates obtained in this study grouped into cluster I, showing no mutual differences, and also grouped with stains JKI4.3.8 from cabbage and C267, Ecc21, C137, Ecc63, M34, and Ecc71 from potato. Strains C144, M30 and A18 originating from potato were placed in cluster II. The other strains of *Pectobacterium* spp. (NCBI) clustered separately: *P. parmentieri* WPP163 and *P. wasabiae* SCRI488 (cluster III), *P. atrosepticum* 36A (cluster IV) and *P. betavasculorum* ATCC43762 (cluster V).

Figure 4 shows the NJ phylogenetic tree made based on partial sequences of the *mdh* gene. The tested cabbage isolates were grouped together with the comparative *P. carotovorum* subsp. *carotovorum* strains Pcct0437, Ecc71 and Ecc21 in cluster I, showing no differences. Strains JKI4.3.8 (cabbage) and C267, C137, Ecc63 (potato) were also placed in cluster I, and showed slight differences from the other cluster members. Similar to the results obtained with the *gapA* gene, other comparative *P. carotovorum* subsp. *carotovorum* strains A18, C144 and M30 (potato) were grouped separately into cluster II. Other *Pectobacterium* spp. strains were separated into clusters III, IV and V. The out-group strain *Y. pestis* was placed on a monophyletic tree branch.

The NJ phylogenetic analysis based on the concatenated sequences of *gapA* and *mdh* genes (Figure 5) also separated the tested cabbage isolates and *P. carotovorum* subsp. *carotovorum* strains retrieved from the NCBI into two tree clusters. Eight cabbage isolates from this study clustered together (cluster I) with the comparative strain JKI4.3.8, also originated from cabbage (Germany), and strains from potato: Ecc21, Ecc71 and Ecc63 (Netherlands) and C267 and C137 (Syria). Strains C144, M30 and A18 originating from potato (Syria) grouped in cluster II. Similar to the results obtained with individual genes (*gapA* and *mdh*), *Pectobacterium* spp. (*P. betavasculorum, P. parmentieri, P. wasabiae*, and *P. atrosepticum*) strains were clearly separated into clusters III, IV, V. The out-group strain *Y. pestis* was grouped separately.

Table 3. Results of biochemical tests obtained for cabbage soft rot isolates

Reaction	Isolates							
	Pcc1	Pcc3	Pcc5	Pcc8	Pcc10	Pcc13	Pcc14	Pcc16
Gram reaction	-	-	-	-	-	-	-	-
Fluorescence on King's B	-	-	-	-	-	-	-	-
Levan formation	-	-	-	-	-	-	-	-
O/F test	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
Arginine dehydrolase	-	-	-	-	-	-	-	-
Oxidase activity	-	-	-	-	-	-	-	-
Catalase activity	+	+	+	+	+	+	+	+
Pectolytic test	+	+	+	+	+	+	+	+

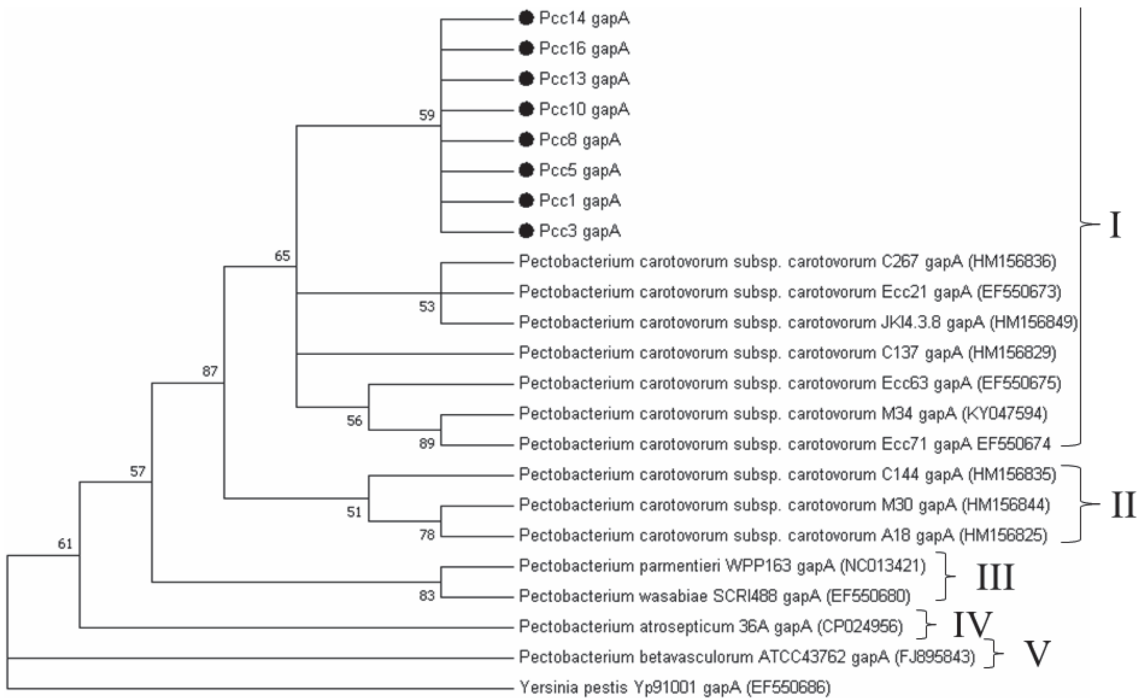

Figure 3. Phylogenetic tree based on *gapA* partial nucleotide sequences showing relations among cabbage isolates and different *Pectobacterium* spp. retrieved from NCBI database

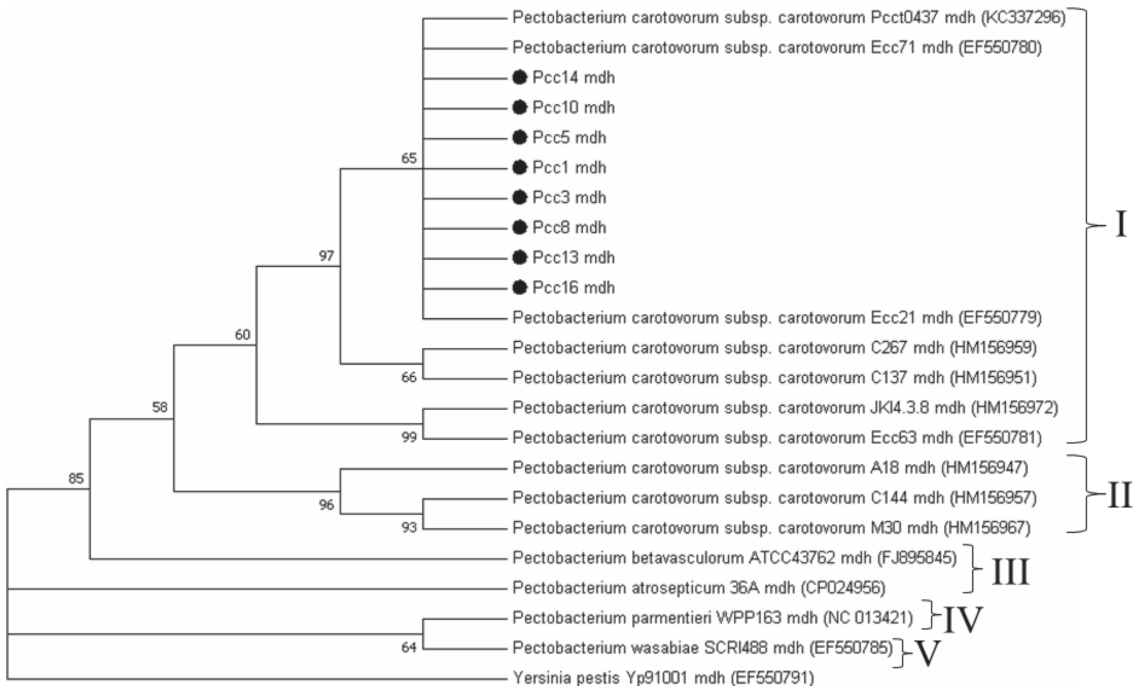

Figure 4. Phylogenetic tree based on *mdh* partial nucleotide sequences showing relations among cabbage isolates and different *Pectobacterium* spp. retrieved from NCBI database

Figure 5. Phylogenetic tree based on concatenated *gapA* and *mdh* nucleotide sequences showing phylogenetic relations among cabbage isolates and different *Pectobacterium* spp. retrieved from NCBI database

The MLSA of *gapA* and *mdh* housekeeping genes determined cabbage isolates to have high similarity with strains M34 and Pcct0437 originating from potato in France and Finland, respectively (Pasanen et al., 2013; Cigna et al., 2017). Partial sequence of the *gapA* gene and set of 35 signature nucleotides allowed to distinguish the species and subspecies from *Pectobacterium* and *Dickeya* genera (Cigna et al., 2017). According to Nabhan et al. (2012b) *P. carotovorum* subsp. *carotovorum* strains obtained from potato in Syria (C267, C137, C144, M30, and A18) and from cabbage in Germany (JKI4.3.8) were divided into the two clusters based on multilocus sequence analysis of eight housekeeping genes (*mtlD, acnA, icdA, mdh, pgi, gabA, proA* and *rpoS*). In our study cabbage isolates from Semberija (Bosnia and Herzegovina) showed high similarity with strains C267, C137 and JKI4.3.8, which were used as comparative. Although *P. carotovora* subsp. *carotovora* strains were divided into different genetic groups, according to Avrova et al. (2002) it does not reflect any obvious subdivision regarding host or geographic origin.

The present study allowed identification of the isolates obtained from soft rotted cabbage originating from Semberija. Since cabbage pathogens from that area have not been studied recently, this survey constitutes a significant contribution to current knowledge of the soft rot pathogen *P. carotovorum* subsp. *carotovorum* on cabbage.

CONCLUSION

In conclusion, soft rot disease that appeared on cabbage in 2018 was determined by conventional bacteriological and molecular tests to have been caused by the pathogen *P. carotovorum* subsp. *carotovorum*. Although using the PCR specific primer pair F0145/E2477 was reliable in rapid identification of all cabbage soft rot isolates, DNA sequencing was the most appropriate method for their characterization. Considering that there is no effective treatment against soft rot bacteria, and that prevention is the only way for their control, early detection and identification of the causing pathogen plays a key role in suppression of disease spread.

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Karakterizacija izolata *Pectobacterium carotovorum* subsp. *carotovorum* sa kupusa izolovanih tokom skorije pojave u Bosni i Hercegovini

REZIME

U radu je identifikovan i okarakterisan prouzrokovač vlažne truleži kupusa u Semberiji. Simptomi su se javljali na listu u vidu lezija vlažnog izgleda uz prisustvo specifičnog mirisa. Bolest je zahvatila 20 do 30% useva. Izolacija patogena prouzrokovača bolesti je vršena na hranljivom agaru (NA), King's B i Kristal violet pektatnoj podlozi (CVP). Za dalja istraživanja odabrano je osam krem-beličastih, okruglih i ispupčenih bakterijskih kolonija koje su stvarale karakteristična udubljenja na CVP podlozi. Svi izolati su bili gram negativni, fakultativni anaerobi, oksidaza negativni, katalaza pozitivni, nefluorescentni na King-ovoj podlozi B, negativni na stvaranje levana i arginin dihidrolaze. Svi izolati su prouzrokovali vlažnu trulež na kupusu i kriškama krompira 24 časa nakon inokulacije u uslovima visoke relativne vlažnosti. Reakcija lančane reakcije polimeraze (PCR) je vršena u cilju preliminarne identifikacije izolata, primenom tri para specifičnih prajmera: F0145/E2477 (specifični za *Pectobacterium carotovorum* subsp. *carotovorum*), Br1f/L1r (specifični za *P. carotovorum* subsp. *brasiliensis*) i ECA1f/ECA2r (specifični za *P. atrosepticum*). Kod svih izolata amplifikovani su produkti veličine 666 baznih parova korišćenjem F0145/E2477 para prajmera, što je ukazalo na prisustvo bakterije *P. carotovorum* subsp. *carotovorum*. Dalja genetička karakterizacija je vršena na osnovu analize sekvenci konzervisanih gena *gapA* i *mdh*. BLAST analiza je potvrdila identifikaciju izolata na osnovu homologije sa sojevima *P. carotovorum* subsp. *carotovorum* deponovanim u NCBI bazi i to od 99.39% (Q. cover 100%, E. value 0.0) sa sojem M34 (Acc.no. KY047594) za sekvence *gapA* gena i 100% (Q. cover 100%, E. value 0.0) sa Pcc t0437 (Acc.no. KC337296) za sekvence *mdh* gena. Filogenetska analiza je pokazala genetičku homogenost izolata poreklom sa kupusa, svrstavajući ih u jedan klaster.

Ključne reči: *Pectobacterium*; kupus; identifikacija; karakterizacija