Arch. Biol. Sci., Belgrade, 65 (2), 585-593, 2013 DOI:10.2298/ABS1302585P

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF *XANTHOMONAS CAMPESTRIS* STRAINS ISOLATED FROM CABBAGE, KALE AND BROCCOLI

TATJANA POPOVIĆ[1*], DRAGANA JOŠIĆ[2], MIRA STAROVIĆ[1], P. MILOVANOVIĆ[3], N. DOLOVAC[1], D. POŠTIĆ[1] and S. STANKOVIĆ[4]

[1] *Institute for Plant Protection and Environment,* 11000 Belgrade, Serbia
[2] *Institute of Soil Science,* 11000 Belgrade, Serbia
[3] *Galenika-Phytopharmacy,* 11000 Belgrade, Serbia
[4] *Faculty of Biology, University of Belgrade,* 11000 Belgrade, Serbia

Abstract - Thirty-six strains of *Xanthomonas campestris* pv. *campestris* (Xcc) isolated from cabbage, kale and broccoli were identified according to their pathogenicity, phenotypic and genotypic characterization. Pathogenicity was confirmed by the injection method with a hypodermic syringe into the mesophilic tissue of cabbage leaves. All strains were Gram-negative, aerobic, catalase-positive, oxidase-negative, grew at 35°C, produced levan, H_2S and indole, did not reduce nitrate, hydrolyzed Tween 80, starch, gelatin and esculin and did not show tolerance to 0.1 and 0.02% TTC. The strains produced acid from d-arabinose, arginine, dulcitol, galactose, d-glucose, maltose, mannose, sorbitol, sucrose and xylose. The genetic characterization was based on the sequence analyses of 16S rDNA and ERIC and BOX PCR. Strains of different pathovars were also used to compare PCR resulting patterns. BOX-PCR of the strains from kale and broccoli, obtained using $(GTG)_5$ primer, yielded patterns with a high similarity level to pathovar reference strain Xcc. The strains from cabbage yielded BOX and ERIC product patterns, distinguishing them from the other tested strains and reference strains. 16S rDNA of the representative strains was closely related to Xcc strain ATCC 33913. ERIC PCR and BOX using $(GTG)_5$ primer generated different Xcc patterns and were effective in distinguishing strains from different plant hosts.

Key words: Xanthomonas campestris, phenotypic and genotypic characterization

INTRODUCTION

Black rot, caused by the bacterium *Xanthomonas campestris* pv. *campestris* (Xcc) is considered the most serious disease of crucifers worldwide (Williams, 1980; Alvarez, 2000). Economically, the most important host is *Brassica oleracea* (including cabbage, cauliflower, broccoli and kale). The disease can cause significant losses, particularly in warm and humid environments (Williams, 1980). Typical symptoms in the field are V-shaped, chlorotic to necrotic lesions starting from leaf margins and blackening of the vascular tissue (Bachi et al., 2008).

Xcc is common worldwide, exhibiting diversity in different countries and different regions of the same country (Vicente et al., 2001; Silva, 2006; Jensen et al., 2010; Popović et al., 2011). Traditionally, the characterization of Xcc has largely depended on biochemical and physiological tests, pathogenicity and phage typing. Variability exists within the pathogen, and differentiation of Xcc strains from other closely related xanthomonads attacking crucifers is often difficult (Massomo et al., 2003) or not possible on the basis of morphological and biochemical characteristics (Franken, 1992).

Recently, monoclonal antibodies and a molecular assay based on the polymerase chain reaction (PCR) were developed for the specific identification of plant pathogenic bacteria (Alvarez et al., 1994; Berg et al., 2005). Genomic fingerprinting by PCR amplification, with primers specific to the highly conserved repetitive extragenic palindromic (REP) sequence, enterobacterial repetitive intergenic consensus (ERIC) and BOX elements, was used successfully to characterize a large number of bacteria and differentiate closely related strains of bacteria, including *Xanthomonas* strains (Scortichini et al., 2003; Rademaker et al., 2005; Valverde et al., 2007). Many strains of *X. campestris* have been characterized by genetic fingerprinting using rep-PCR (Massomo et al., 2003; Tsygankova et al., 2004; López et al., 2006; Valverde et al., 2007; Zaccardelli et al., 2008; Jensen et al., 2010). Ignatov et al. (2007) used amplified fragment length polymorphism (AFLP) to assess the genetic diversity among strains of *X. campestris* in weeds in non-cultivated and cultivated areas. AFLP and multilocus sequence analysis (MLSA) was consistently achieved for identification of xanthomonads at the species and pathovar levels (Ah-You et al., 2009). PFGE has been used to determine variability among different *Xanthomonas* strains (Valverde et al., 2007).

During 2010, Xcc strains were collected from cabbage, kale and broccoli (the Novi Sad area 45°15' N and 19°50' E) in order to estimate their phenotypic and genotypic diversity. Phenotypic methods were applied to the investigated strains and pathovar reference strain NCPPB 1144, and genotypic methods were used for comparison with Xcc strains of different pathovars (*campestris*, *pruni*, *pelargonii*, *fragariae*).

MATERIALS AND METHODS

Collection of leaf samples

Samples of plants showing black rot symptoms were collected between July and September 2010 from fields with diseased Brassica crops (cabbage, *Brassica oleracea* var. *capitata*; kale, *B.o.* var. *acephala* and broccoli, *B. oleracea* var. *botrytis*) for the isolation of bacteria. From each field, infected leaves were placed in paper bags and transported to the laboratory for further processing. The leaves were washed with sterile distilled water and dried at room temperature (25°C) on absorbent paper before the isolation of causal bacteria associated with black rot symptoms.

Bacterial isolation

From each sample, leaf tissue segments were excised from the lesion margins. The leaf segments were macerated in sterile distilled water and left to stand for 5 min. Bacteria were isolated on nutrient agar plates (NA). After two days of incubation at 30°C, yellow, translucent, circular, and raised colonies were transferred to yeast dextrose chalk medium (YDC). Plates were inspected for the presence of pale yellowish, convex, mucoid bacterial colonies two days after incubation at 28°C. Suspected single colonies were selected and purified by re-streaking on YDC medium. Thirty-six strains were included in this study. Xcc strain NCPPB 1144 (National Collection of Plant Pathogenic Bacteria, UK) was used as a reference strain.

Pathogenicity

Pathogenicity was confirmed by inoculating cabbage leaves by the injection method with a hypodermic syringe into the mesophyll tissue of cabbage leaves and petioles with bacterial inoculum, grown on YDC agar for 48 h at 28°C, harvested and adjusted to 10^8 cfu/ml in sterile distilled H_2O. The leaves were incubated for 24 h in humid growth chambers maintained at 25°C with a 16/8 h light regime. Thereafter, they were removed from the humid chambers and kept under the same light and temperature conditions. The types of symptoms induced were recorded 5-7 days after inoculation. The following three tests were chosen for further characterization of the strains.

Phenotypic characterization

All strains of Xcc were characterized by the methods of Dye (1962) and Lelliot and Stead (1987). For all tests, cultures were grown on YDC at 28°C for 48 h.

Oxidative/fermentative (OF) medium supplemented with glucose was used to determine the type of metabolism for each strain. Strains were Gram-stained and tested for Kovacs' oxidase reaction, catalase production, levan, nitrate reduction, H_2S production, indole production, starch hydrolysis, gelatin hydrolysis, esculin hydrolysis, Tween 80 lipolysis, growth at 35°C, tolerance to 0,1 and 0.02% triphenyl-tetrazolium chloride (TTC) and acid production from d-arabinose, arginine, dulcitol, galactose, d-glucose, maltose, mannose, sorbitol, sucrose and xylose.

PTA ELISA

Strains suspected to be Xcc were tested using polyclonal antibodies (ADGEN Phytodiagnostics, Neogen Europe) following the manufacturer's instructions. Bacterial suspensions (3×10^8 cfu ml^{-1}) were prepared in sterile water with pure bacterial cultures grown on the YDC for 48 h at 27°C.

Genetic characterization
DNA isolation

DNA was extracted from cultures grown for 24 h on YDC by using the protocol described by Ross et al. (2000). DNAs from the strains listed in Table 1 were used as templates to estimate their diversity and for 16S rDNA sequences analysis. DNAs from the strains Xcc (NCPPB 1144), *X. arboricola* pv. *pruni* (NCPPB 3156), *X. hortorum* pv. *pelargonii* (NCPPB 3330) and *X. fragariae* (NCPPB 2473) were used and run next to the investigated samples on agarose gels for the comparison. The sizes of fragments were determined by comparison with DNA molecular weight marker GeneRuler DNA Ladder mix SM0331 (Fermentas, Lithuania). All amplifications were carried out using DreamTaqGreen Master Mix (Fermentas, Lithuania) and Eppendorf Mastercycler Personal thermocycler (Germany).

BOX and ERIC PCR

A total of 36 strains obtained from the three host-plants were analyzed. PCRs were carried out in 25µl mixtures containing DreamTaqGreen Master Mix and 30 pM primer $(GTG)_5$ for BOX analysis and 50 pM ERIC1R/ERIC2 primer set (Versalovic et al., 1991). The amplification conditions consisted of an initial denaturation at 94°C for 3 min, 35 cycles at 94°C for 1 min, at 53°C for 1 min, and at 65°C for 8 min; the final extension was at 65°C for 16 min for $(GTG)_5$; ERIC PCRs were carried out using an initial step of 95°C for 2 min and annealing at 52°C for 1 min. The PCR products were analyzed by 1.5% agarose gel electrophoresis in 0.5x TBE buffer for 3 h at 90 V and were stained with ethidium bromide.

Data analysis.

BOX and ERIC PCR fingerprint results were converted to binary form and cluster analysis of strains were done with the program STATISTICA 7.

16S rDNA analysis

Primers fD1 and rD1 (Weisburg et al., 1991), derived from the conserved regions of 16S rRNA genes and capable to amplify nearly full-length 16S rDNA from many bacterial genera, were used for amplification. The following temperature profile was used: initial denaturation at 95°C for 3 min, 35 cycles of denaturation (1 min at 94°C), annealing (1 min at 57°C), extension (2 min at 72°C) and final extension at 72°C for 6 min. After horizontal electrophoresis in 1.2% agarose, PCR products were purified using PCR purification kit or Gel Extraction kit (Fermentas, Lithuania).

Nucleotide sequence accession numbers

The nucleotide sequences of representative strains determined in this study using the facility of IMGGI SeqService (Belgrade) have been deposited in the GenBank database under accession numbers JQ818431, JQ818432, JQ818433.

RESULTS

In this research, we characterized Xcc strains isolated from the V-shaped lesions typical of black rot from cabbage, kale and broccoli. After 48 h incubation, the

Table 1. Bacterial strains used in study

Strains	Host	Location	Date isolated
TKU1 - TKU5	Cabbage	Rimski Šančevi 45°32´ N, 19°83´ E	Aug. 2010
TKU6 - TKU15	Cabbage	Rimski Šančevi 45°32´ N, 19°83´ E	Sep. 2010
TKE1 - TKE3	Kale	Rimski Šančevi 45°32´ N, 19°83´ E	Jul. 2010
TKE4 - TKE9	Kale	Rimski Šančevi 45°32´ N, 19°83´ E	Aug. 2010
TBR1 - TBR5	Broccoli	Rimski Šančevi 45°32´ N, 19°83´ E	Jul. 2010
TBR6 - TBR12	Broccoli	Rimski Šančevi 45°32´ N, 19°83´ E	Aug. 2010

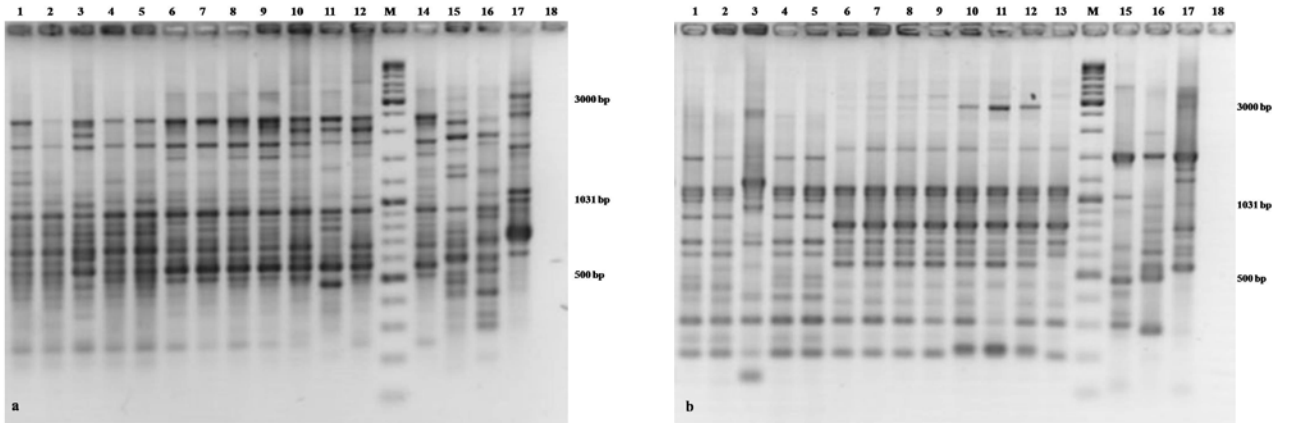
Table 2. Biochemical characteristics of *Xanthomonas campestris* pv. *campestris* strains.

Tests	Reaction	
	Investigated strains	Reference strain NCPPB 1144
Oxidase reaction	-	-
Catalase reaction	+	+
Levan production	+	+
Oxidative metabolism of glucose	+	+
Nitrate reduction	-	-
H_2S production	+	+
Indole production	+	+
Starch hydrolysis	+	+
Gelatin hydrolysis	+	+
Esculin hydrolysis	+	+
Tween 80 lipolysis	+	+
Growth at 35°C	+	+
Tolerance of 0,1 and 0,02% TTC	-	-
Acid produce from:		
d-arabinose	+	+
arginine	+	+
dulcitol	+	+
galactose,	+	+
d-glucose	+	+
maltose	+	+
mannose	+	+
sorbitol	+	+
sucrose	+	+
xylose	+	+

colonies were small, yellow on NA or round, convex, mucoid, yellow on YDC medium. Thirty-six representative strains were selected – 15 from cabbage, 9 from kale and 12 from broccoli (Table 1).

The identity of the strains was confirmed by the pathogenicity test. All strains induced symptoms after 3-5 DAI, caused dark green water-soaking spots, followed by yellowing and the collapse of the inoculated tissue.

The bacterial strains were subjected to various biochemical and physiological tests. The results showed that all strains were Gram-negative, aerobic, catalase-positive and oxidase-negative. The strains grown at 35°C produced levan, H_2S and indole, did not reduce nitrate, hydrolyzed Tween 80, starch, gelatin and esculin and did not show tolerance to 0.1 and 0.02% TTC (Table 2). The strains produced acid from d-arabinose, arginine, dulcitol, galactose, d-glucose, maltose, mannose, sorbitol, sucrose and



(a) (b)

Fig. 1. Rep-PCR of indigenous strains *Xanthomonas campestris* pv. *campestris*. a) BOX PCR obtained by $(GTG)_5$ primer: Lanes 1-5: strains from cabbage TKU1, TKU4, TKU7, TKU9, TKU14; Lanes 6-9: strains from broccoli TBR2, TBR5, TBR8, TBR11; Lanes 10-12: strains from kale TKE3, TKE5, TKE9. Lane 13. Marker; 14. Xcc (NCPPB 1144); 15. *X. arboricola* pv. *pruni* (NCPPB 3156); 16. *X. hortorum* pv. *pelargonii* (NCPPB 3330); 17. *X. fragariae* (NCPPB 2473); 18. negative control b) ERIC PCR: Lanes 1-5: strains from cabbage TKU1, TKU4, TKU7, TKU9, TKU14; Lanes 6-9: strains from broccoli TBR2, TBR5, TBR8, TBR11; Lanes 10-12: strains from kale TKE3, TKE5, TKE9. Lane 13. Xcc (NCPPB 1144); 14. Marker; 15. *X. arboricola* pv. *pruni* (NCPPB 3156); 16. *X. hortorum* pv. *pelargonii* (NCPPB 3330); 17. *X. fragariae* (NCPPB 2473); 18. negative control. Marker- GeneRuler DNA Ladder mix SM0331 (Fermentas, Lithuania).

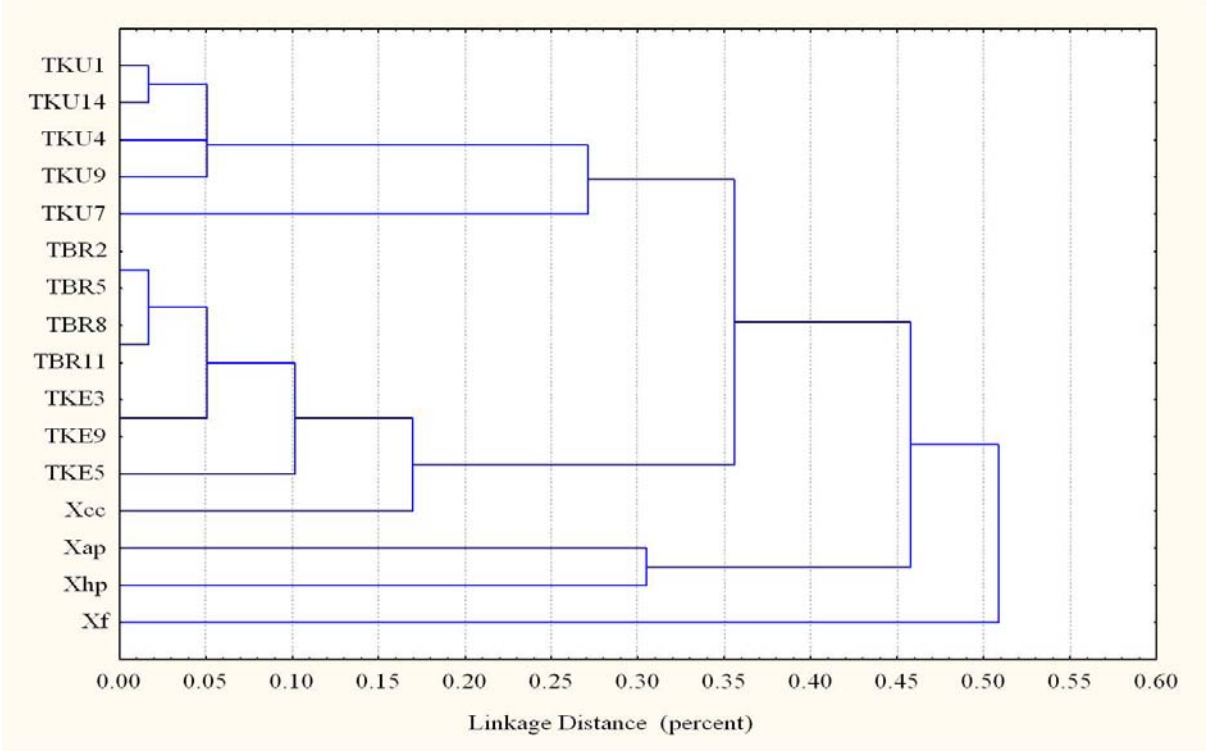


Fig. 2. Dendrogram of genetic similarity of *Xanthomonas campestris* pv. *campestris* strains from cabbage, kale and broccoli based on combined BOX with $(GTG)_5$ primer and ERIC fingerprinting data.

xylose (Table 2). All strains indicated uniform reactions in the tests used as a Xcc reference strain.

All strains were tested in PTA ELISA using the polyclonal antibodies (ADGEN) specific to the Xcc pathogen. All strains reacted with the used Xcc-specific antibodies. According to the serological test of Xcc strains with respect to their reactivity to antibodies, the strains belong to Xcc bacterium.

PCR using DNA primers corresponding to conserved motifs in bacterial repetitive elements (rep-PCR) were used to obtain genomic fingerprints of Xcc. Both analyses yielded a complex fingerprint pattern with bands ranging between 150 and 3000 bp. The strains from kale and broccoli generated two very similar patterns and showed high similarity to the pathovar reference strain Xcc (NCPPB 1144) (Fig. 1). Strains from cabbage yielded BOX and ERIC product patterns, distinguishing them from other tested strains and reference strains.

Cluster analysis of the data obtained from applied PCRs on the investigated strains revealed two major clusters (Fig. 2). One cluster included all strains from kale and broccoli and the type strain Xcc (NCPPB 1144), and the other cluster contained all strains from cabbage. The mean similarity level between the clusters was about 65%. Low similarity (48-54%) and separate clusters were observed with strains *X. arboricola* pv. *pruni* (NCPPB 3156), *X. hortorum* pv. *pelargonii* (NCPPB 3330) and *X. fragariae* (NCPPB 2473). Within the cluster formed by the strains from kale and broccoli, similarity levels (90%) were observed. In the cluster from the cabbage strains, one strain was distinguished from the group at a mean level of similarity of about 73%.

Nearly full-length 16S rDNA (1504-1510 bp) from the strains was amplified using an fD1/rD1 primer set. Partial sequence analysis of rDNA revealed high levels of similarity among the TKU7 from cabbage, TBR11 from broccoli and TKE5 from kale, as representative strains. Results were compared with similar sequences in NCBI and showed high similarity (99%) to Xcc strain ATCC 33913.

DISCUSSION

The present study describes the occurrence of black rot in Serbia crucifers and the diversity of the causal bacterium Xcc. Symptoms of V-shaped leaf lesions with black veins were observed in all visited fields, and Xcc was isolated from these lesions from cabbage, kale and broccoli. Symptoms characterized by dark green water-soaking spots, followed by yellowing and the collapse of the inoculated tissue, were observed on inoculated cabbage leaves. Necrosis and mesophyll tissue collapse in advance of blackening of veins were described by Alvarez et al. (1994). According Williams (1980) and Alvarez (2000), blackened vascular tissues and V-shaped chlorotic-to-necrotic lesions along the leaf margins on the true leaves are the characteristic symptoms of black rot. The symptoms in cabbage depend upon several factors such as cultivar, plant age (Schaad and Alvarez, 1993), light and temperature, humidity, the strain of the pathogen and even the method used for inoculation (Franken, 1992).

Our strains were subjected to various biochemical and physiological tests and results showed similarity with the literature description for Xcc given by Schaad and Alvarez (1993), Swings et al. (1993) and Alvarez et al. (1994). The results also suggest that the strains appear to be phenotypically similar.

Serological assays are useful in the confirmation test for the isolated colonies or for direct detection from seed (Franken, 1992) or leaves (Alvarez and Lou, 1985). Serology with monoclonal antibodies is particularly specific for the identification or differentiation of Xcc strains and has been used successfully in monitoring of the bacterium in the fields (Yuen et al., 1987; Alvarez et al., 1994). In this study, the identity of Xcc strains was confirmed by PTA ELISA using the polyclonal antibodies.

Different fingerprints were generated by the products of $(GTG)_5$ and ERIC PCRs. The investigated strains from broccoli, kale and the strain Xcc NCPPB 1144 showed a similar pattern overall. Polymorphic bands were also observed, and two patterns could

be differentiated with a similarity level between 90 and 95%. Strains from cabbage generated different patterns with one major group of strains and one separate strain TKU7. Applied $(GTG)_5$ primer as a BOX generated more differences in the patterns of observed strains than BOXA1R primer (data not shown). In addition, this primer was useful for distinguishing very similar kale and broccoli strains.

For *Xanthomonas* species, rep-PCRs have been used to assess variation among pathovars and have revealed low levels of intrapathovar diversity (Louws et al., 1994; Louws et al., 1999). According to Louws et al. (1994), intrapathovar diversity in *Xanthomonas* spp. could be grouped as pathovars from which the strains had almost identical fingerprints or had overall unique profiles but shared multiple bands, or pathovars from which the strains could be divided into groups and did not share common banding patterns. In our investigation, the strains from cabbage, kale and broccoli were found to belong to the first category.

López et al. (2006) estimated differences in the ability of primers to reveal diversity in *Xanthomonas* spp. and genetic diversity of *X. axonopodis* pv. *phaseoli* and its var. *fuscans*. They reported that ERIC-PCR revealed more polymorphic bands among the strains than the other two sets of rep-PCR primers. Valverde et al. (2007) compared rep-PCR with PFGE and AFLP for their potential to assess the strain diversity of Xcc and showed that these techniques possess comparable resolution capabilities to assess the diversity within this pathogen.

Analysis of the 16S rDNA sequences of all *Xanthomonas* species revealed very limited diversity (Hauben et al., 1997). Investigated strains of Xcc in our study showed a high level of 16S rDNA similarity between Xcc strain ATCC 33913.

The applied rep-PCR fingerprinting using ERIC1R/2 and $(GTG)_5$ primers was confirmed as a useful tool for the fast estimation of diversity and for the first step of monitoring of this pathogen in the affected areas. Additional analysis should be done for better characterization of the representative strains with different patterns and for comparison with Xcc from other host plants.

The combined results of this genetic study, together with those of morphological, biochemical and serological analyses, point to the fact that strains from cabbage, kale and broccoli belong to the species Xcc. Results suggest that Xcc strains showed uniformity in all used tests as a reference strain. The PCR analysis suggested that the tested strains from broccoli and kale showed low polymorphisms and a higher level of similarity to the reference strain Xcc (NCPPB 1144) compared to BOX and ERIC patterns of Xcc strains from cabbage. 16S rDNA of the representative isolates was closely related to the Xcc strain ATCC 33913. ERIC PCR and BOX using $(GTG)_5$ primer generated different Xcc patterns and were effective in distinguishing the strains from different plant hosts growing in the Serbian geographic area.

Since the Xcc strains were characterized with phenotypic and genotypic methods in this study, we can compare these results with those for strains from other localities and host plants in future investigations.

Acknowledgments - The work is a part of the Projects No. III43010 and III46007 funded by the Ministry of Education, Science and Technological Development, Republic of Serbia.

REFERENCES

Ah-You, N., Gagnevin, L., Grimont, P.A., Brisse, S., Nesme, X., Chiroleu, F., Bui Thi Ngoc, L., Jouen, E., Lefeuvre, P., Vernière, C., and *O. Pruvost* (2009). Polyphasic characterization of xanthomonads pathogenic to Anacardiaceae and their relatedness to different *Xanthomonas* species. *International Journal of Systematic and Evolutionary Microbiology* **59**, 306-318.

Alvarez, A., Benedict, A.A., Mizumoto, C.Y., Hunter, J.E., and *D.W. Gabriel* (1994). Serological, pathological, and genetic diversity among strains of *Xanthomonas campestris* infecting crucifers. *Phytopathology* **81**, 1449-1457.

Alvarez, A.M. (2000). Black rot of crucifers, In: *Mechanisms of Resistance to Plant Diseases* (Eds. A.J. Slusarenko, R.S. Fraser, and L.C. van Loon,), 21-52. Kluwer Academic Publishers, Dordrecht The Netherlands.

Alvarez, A.M., and *K. Lou* (1985). Rapid identification of *Xanthomonas campestris* pv. *campestris* by ELISA. *Plant Disease* **69**, 1082-1086.

Berg, T., Tesoriero, L.A., and *D.L. Hailstones* (2005). PCR-based detection of *Xanthomonas campestris* pathovars in Brassica seed. *Plant Pathology* **54**, 416-427.

Dye, D.W. (1962). The Inadequacy of the Usual Determinative Tests for the Identification of *Xanthomonas* spp. *New Zealand Journal of Science* **5**, 393-416.

Franken, A.A.J.M. (1992). Comparison of immunofluorescence microscopy and dilution-plating for the detection of *Xanthomonas campestris* pv. *campestris* in crucifer seeds. *Netherlands Journal of Plant Pathology* **98**, 169-178.

Hauben, L., Vauterin, L., Swings, J., and *E.R.B. Moore* (1997). Comparison of 16S ribosomal DNA sequences of all *Xanthomonas* species. *International Journal of Systematic Bacteriology* **47**, 328-335.

Ignatov, A., Sechler, A., Schuenzel, E.L., Agarkova, I., Oliver, B., Vidaver, A.K., and *N.W. Schaad* (2007). Genetic diversity in populations of *Xanthomonas campestris* pv. *campestris* in cruciferous weeds in central coastal California. *Phytopathology* **97**, 803-812.

Jensen, B.D., Vicente, J.G., Manandhar, H.K., and *S.J. Roberts* (2010). Occurrence and diversity of *Xanthomonas campestris* pv. *campestris* in vegetable *Brassica* fields in Nepal. *Plant Disease* **94**, 298-305.

Lelliott, R.A., and *D.E. Stead* (1987). *Methods for the diagnosis of bacterial diseases of plants*. Blackwell Scientific Publications, Oxford, UK.

López, R., Asensio, C., and *Gilbertson, R.L.* (2006). Phenotypic and genetic diversity in strains of common blight bacteria (*Xanthomonas campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans*) in a secondary center of diversity of the common bean host suggests multiple introduction events. *Phytopathology* **96**, 1204-1213.

Louws, F.J., Fulbright, D.W., Stephens, C.T., and *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**, 2286-2295.

Louws, F.J., Rademaker, J.L.W., and *F.J. De Bruijn* (1999). The three Ds of PCR-based genomic analysis of phytobacteria: diversity, detection, and disease diagnosis. *Annual Review of Phytopathology* 37, 81-125.

Massomo, S.M.S., Nielsen, H., Mabagala, R.B., Mansfeld-Giese, K., Hockenhull, J., and *C.N. Mortensen* (2003). Identification and characterisation of *Xanthomonas campestris* pv. *campestris* strains from Tanzania by pathogenicity tests, Biolog, rep-PCR and fatty acid methyl ester analysis. *European Journal of Plant Pathology* **109**, 775-789.

Opgenorth, D.C., Smart, C.D., Louws, F.J., De Bruijn, F.J., and *B.C. Kirkpatrick* (1996). Identification of *Xanthomonas fragariae* field isolates by rep-PCR genomic fingerprintings. *Plant Disease* **80**, 868-873.

Pooler, M.R., Ritchie, D.F., and *J.H. Hartung* (1996). Genetic relationships among strains of *Xanthomonas fragariae* based on random amplified polymorphic DNA PCR, repetitive extragenic palindromic PCR, and enterobacterial repetitive intergenic consensus PCR data and generation of multiplexed PCR primers useful for the identification of this phytopathogen. *Applied and Environmental Microbiology* **62**, 3121-3127.

Popović, T., Jošić, D., Starović, M., Aleksić, G., Poštić, D., Stajković, O., and *M. Mijatović* (2011). Genetic diversity of *Xanthomonas campestris* pv. *campestris* isolated from cabbage, kale and broccoli. *Proceedings of the 7th Balkan Congress of Microbiology - Microbiologia Balkanica*. Belgrade Serbia.

Rademaker, J.L.W., Louws, F.J., Schultz, M.H., Rossbach, U., Vauterin, L., Swings, J., and *F.J. De Bruijn* (2005). A comprehensive species to strain taxonomic framework for *Xanthomonas*. *Phytopathology* **95**, 1098-1111.

Ross, L.I., Alami, Y., Harvey, R.P., Achouak, W., and *M.H. Ryder* (2000). Genetic diversity and biological control activity of novel species of closely related pseudomonads isolated from wheat field soils in South Australia. *Applied and Environmental Microbiology* **66**, 1609-1616.

Schaad, N.W., and *A. Alvarez* (1993). *Xanthomonas campestris* pv. *campestris*: cause of black rot of crucifers, In: Xanthomonas (Eds. J.S. Swings, and E.L. Civerolo), 51-56. Chapman and Hall, London, UK.

Scortichini, M., and *M.P. Rossi* (2003). Genetic diversity of *Xanthomonas arboricola* pv. *fragariae* strains and comparison with some other *X. arboricola* pathovars using repetitive PCR genomic fingerprinting. *Journal of Phytopathology* **151**, 113-119.

Seebold, K., Bachi, P., and *J. Beale* (2008). Black rot of crucifers. UK Cooperative Extension Service. University of Kentucky. Available at: http://www.ca.uky.edu/agcollege/plantpathology/ext_files/PPFShtml/PPFS-VG-1.pdf. Accessed on February, 2008.

Shigaki, T., Nelson, S.C., and *A.M. Alvarez* (2000). Symptomless spread of blight-inducing strains of *Xanthomonas campestris* pv. *campestris* on cabbage seedlings in misted seedbeds. *European Journal of Plant Pathology* **106**, 339-346.

Silva, M.R. (2006). Genetic diversity of *Xanthomonas campestris* pv. *campestris* in Brazil. Master's Thesis. Universidade Federal de Viçosa. Brazil.

Swings, J., Vauterin, L., and *K. Kersters* (1993). The bacterium *Xanthomonas*, In: *Xanthomonas* (Eds. J.S. Swings, and E.L. Civerolo), 121-156. Chapman and Hall, London, UK.

Tsygankova, S.V., Ignatov, A.N., Boulygina, E.S., Kuznetsov, B.B., and *E.V. Korotkov* (2004). Genetic relationships among strains of *Xanthomonas campestris* pv. *campestris* revealed by novel rep-PCR primers. *European Journal of Plant Pathology* **110**, 845-853.

Valverde, A., Hubert, T., Stolov, A., Dagar, A., Kopelowitz, J., and *S. Burdman* (2007). Assessment of genetic diversity of *Xanthomonas campestris* pv. *campestris* isolates from Israel by various DNA fingerprinting techniques. *Plant Pathology* **56**, 17-25.

Versalovic, J., Koeuth, T., and *J.R. Lupski* (1991). Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting bacterial genomes. *Nucleic Acids Research* **19**, 6823-6831.

Vicente, J.G., Conway, J., Roberts, S.J., and *J.D. Taylor* (2001). Identification and origin of *Xanthomonas campestris* pv. *campestris* races and related pathovars. *Phytopathology* **91**, 492-499.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., and *D.J. Lane* (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology* **173**, 697-703.

Williams, P.H. (1980). Black rot: A continuing threat to world crucifers. *Plant Disease* **64**, 736-742.

Yuen, G.Y., Alvarez, A.M., Benedict, A.A., and *K.J. Trotter* (1987). Use of monoclonal antibodies to monitor the dissemination of *Xanthomonas campestris* pv. *campestris*. *Phytopathology* **77**, 366-370.

Zaccardelli, M., Campanile, F., Moretti, C., and *R. Buonaurio* (2008). Characterization of Italian populations of *Xanthomonas campestris* pv. *campestris* using primers based on DNA repetitive sequences. *Journal of Plant Pathology* **90**, 375-381.