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ERIC PCR AS A METHOD FOR DETERMINING DIVERSITY OF *XANTHOMONAS ARBORICOLA* PV*. JUGLANDIS*

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INTRODUCTION

The Persian walnut (*J. regia* L.) is widely cultivated and important commercial nut. Walnut, bacterial blight is considered as the most important one in all walnut-growing areas. *Xanthomonas arboricola* pv. *juglandis* is the causal agent of walnut blight. Disease affects leaves, fruits, twigs and branches and may cause severe losses of fruits. The main field symptom is sudden wilting of the twigs, branches, and tree, especially at the end of spring and in the summer.
Genomic fingerprinting of the *Xanthomonas arboricola* pv. *juglandis* populations by repetitive PCR performed with ERIC primer set could reveal a level of diversity among the populations of this bacterium in Serbia.

Figure 1. walnut blight symptoms caused by *Xanthomonas arboricola* pv. *juglandis*

MATERIAL AND METHODS

The *X. arboricola* pv. *juglandis* isolates that were used in this study were collected from different areas of walnut cultivation in Serbia. Symptomatic samples were taken from buds, leaf and fruit surface and isolation of the pathogen has been cultured on YDC medium. Total genomic DNA was prepared by using a modification of the procedure of Ausubel et al. (1992). Amplification was performed with a ERIC1R-I and ERIC2 primers. The primers were a sequences: (ERIC1R [59-ATGTAAGCTCCTGGGGATTCAC-39] and ERIC2 [59-AAGTA AGTGACTGGGGTGAGCG-39]). The PCR protocols with ERIC primers are referred to as ERIC-PCR.

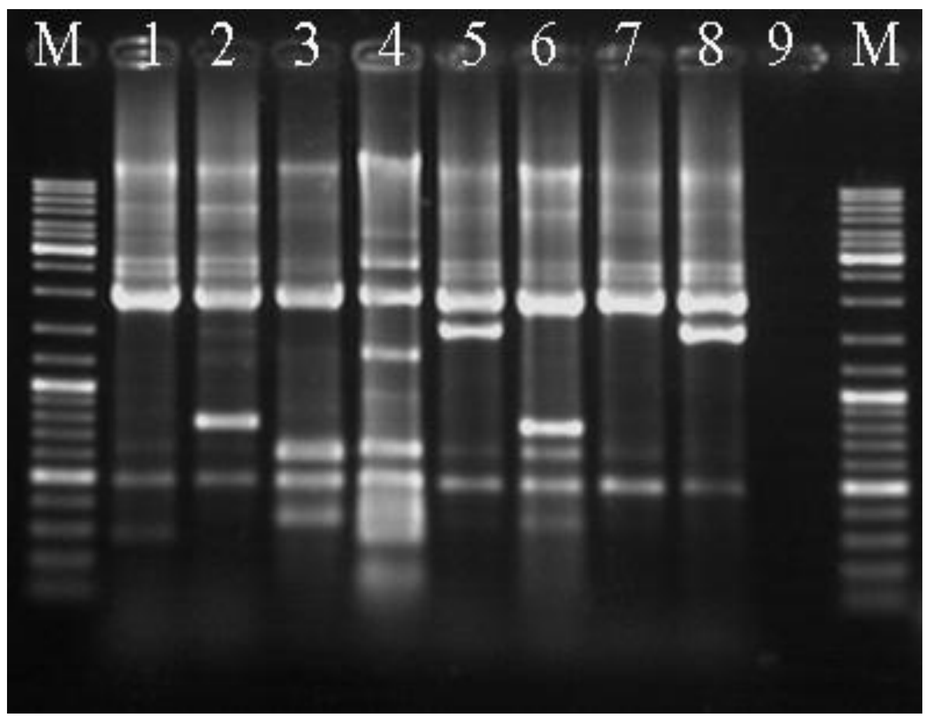

Figure 2. Agarose gel electrophoresis of repetitive-sequence – based polymerase chain reaction (ERIC-PCR) fingerprint patterns obtained from *X. arboricola* pv. *juglandis.* CFBP-2528 (lanes 1), CFBP-2567 (lanes 2), *X. arboricola* pv. *juglandis* isolate from fruit, Ruma (lane 3), *X. arboricola* pv. *juglandis* isolate from leaf, Ratkovo (lane 4-6), *X. arboricola* pv. *juglandis* isolate from fruit Ratkovo (lane 7-8), negative control (lane 9); DNA molecular size marker (GeneRulerTM DNA Ladder Mix) (lane M).

RESULTS

Positive isolation occurs in most cases from leaf and fruit surface. These isolates formed yellow-colored mucoid and convex colonies after 3 days of incubation at 28° C. ERIC primer sets gave reproducible genomic PCR profiles consisting of bands ranging in size from approximately 100 bp to over 6 kb. With the ERIC primers distinct DNA polymorphism was observed in the region between 100 and 3 kb. ERIC-PCR analyses indicated genetic diversity among the isolates which could be divided in four groups with the different types of profile.

DISCUSSION

The purpose of the present study was to investigate the genetic diversity of *X. arboricola pv. juglandis.* This bacterium has been identified as the causal agent of a known disease on walnut - bacterial blight. Pathogenicity tests demonstrated that *X. arboricola* pv. *juglandis* isolates caused infections on fruits and leaves. In the study we ascertained that *X. arboricola* pv. *juglandis* could be divided in four groups currently causing disease on walnut in Serbia. The results obtained with genetic techniques clearly differentiate four populations of this bacterium. This indicates that each area of Persian walnut cultivation has a different *X. arboricola* pv. *juglandis* population. In this study it has been shown the potential of ERIC PCR as a diagnostic tool in determining the genetic difference between *X. arboricola* pv. *juglandis* populations.