CHARACTERIZATION OF *PSEUDOMONAS SYRINGAE* STRAINS BY ERIC PCR GENOMIC FINGERPRITING

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INTRODUCTION

The most widespread and economically important plant pathogen *Pseudomonas syringae* is found on numerous hosts including fruit trees, field crops, vegetables and decorative plants. *P. syringae* causes the blossom blast, shoot blight, bud necrosis, branchies decay and dying of whole trees. Identification of the bacterium is accurate using the pathogenicity test and biochemical characteristics. The pathogen has the ability to kill both young and older trees. The occurrence of frost injury or exposure to freezing temperatures is important predisposition factor in canker development by *P. syringae*. To estimate possible diversity of *P. syringae* fruit trees strains, we collected a set of isolates in several areas of Serbia. The samples were isolated from infected orchards of raspberry, plum, cherry, sour cherry, peach, pear and apple trees. This study was undertaken to characterize the genetic diversity of *P. syringae* strains from a fruit trees, using ERIC-PCR. The genomic DNA fingerprinting technique known as repetitive sequence – based polymerase chain reaction (rep-PCR) was utilized as a tool to differentiate *P. syringae* strains isolated from the different plant hosts. ERIC DNA primers, were used to generate genomic fingerprints. The ERIC-PCR generated patterns of DNA fragments were observed after the agarose gel electrophoresis.

MATERIAL AND METHODS

Pseudomonas syringae were isolated between 2008. and 2010. from pear, peach, apple, plum, cherry, sour cherry and raspberry trees, originating from different localities in Serbia. As the check strains CFBP 1582 (*P. syringae* pv. *syringae*) and CFBP 2119 (*P. syringae* pv. *morprunorum*) from the French collection of phytopathogenic bacteria were used in this work. Pathogenic characteristics of the isolates were tested by artificial inoculation of pear, and cherry fruit. In order to check the hypersensitive reaction (HR), *Geranium* leaves were inoculated with the bacterial suspension of 10^7 cfu/ml. Total genomic DNA was prepared by using a modification of the procedure of Ausubel et al. (1992). Amplification was performed with the primers (ERIC1R [59-ATGTAAGCTCCTGGGGATTCAC-39] and ERIC2 [59-AAGTA AGTGACTGGGGTGAGCG-39]). The PCR conditions were as previously described (de Bruijn, 1992). The PCR protocols with ERIC primers are referred to as ERIC-PCR. Fingerprints generated from different strains were compared visually.

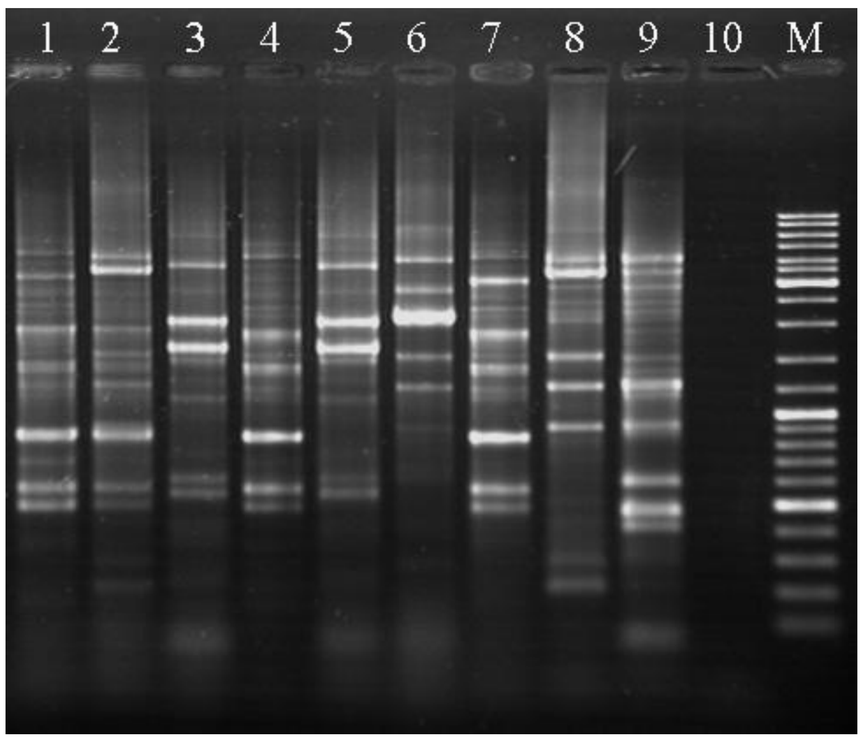

Fig. 1. Agarose gel electrophoresis of as repetitive-sequence – based polymerase chain reaction (ERIC-PCR) fingerprint patterns obtained from *Pseudomonas syringae.* CFBP-1582 (lane 1), CFBP-2119 (lane 2), *P. syringae* isolate from plum (lane 3), *P. syringae* isolate from sour cherry (lane 4), *P. syringae* isolate from cherry (lane 5), *P. syringae* isolate from pear (lane 6), *P. syringae* isolate from apple (lane 7); *P. syringae* isolate from raspberry (lane 8), *P. syringae* isolate from peach (lane 9) and negative control (lane 10); DNA molecular size marker (GeneRulerTM DNA Ladder Mix) (lane 11).

RESULTS

The investigated isolates showed significant heterogeneity in pathogenic characteristics. All of them cause hypersentivity (HR) on tobacco, but in other respects the isolates behave differently. The isolates from peach, pear, apple, sour cherry and raspberry cause necrosis on inoculated unripe pear and cherry, demonstrating typical characteristics of *P. syringae* pv. *syringae*. The isolates from necrotic plum and cherry buds, have caused necrosis of cherry fruit but without effects on pear, showing characteristic of the *P. syringae* pv. *morsprunorum.* In this study, the *P. syringae* pv. *syringae* strains isolated from pear, peach, apple, sour cherry and raspberry trees, generated different genetic profiles in ERIC-PCR whereas strains of *P. syringae* pv. *morsprunorum* isolated from plum and cherry hosts, generated similar patterns. This suggests a host specialization of the stone fruit strains within the heterogeneous pathovar *syringae.* The rep-PCR genomic fingerprints generated with the ERIC primer from the 10 virulent isolates enabled us to distinguish among the different strains of *P. syringae*. The fingerprint patterns of *P. syringae* strains are shown in Fig. 1. Fingerprint profiles generated with ERIC primer were complex and very different among the isolates. The ERIC - PCR yielded 5 to 15 distinct PCR products, ranging in size from approximately 100 bp to over 6 kb. Differences among strains were assessed visually on the basis of the migration patterns of the PCR products.

DISCUSSION

Our research strategy was based on the finding that *P. syringae* was associated with a number of strains that parasites the stone fruit trees in Serbia. All of bacterial isolates cause hypersentivity (HR) on tobacco and *Geranium* leaves. They showed heterogeneity in pathogenic characteristics, causing necrosis on inoculated unripe pear and cherry. Concerning their pathogenic characteristics, the investigated strains belong to two distinct groups. The first one contains varieties originating from peach, pear, apple, sour cherry and raspberry demonstrating typical characteristics of *P. syringae* pv. *syringae*. The other group comprises isolates from cherry and plum buds, showing characteristics tyical for *P. syringae* pv. *morsprunorum*. Detection of differences among *P. syringae* strains was successfully performed using ERIC-PCR method. This kind of characterization was first time used to discriminate *P. syringae* isolates originating from fruit trees in Serbia. Genetic fingerprints were determined for strains of *P. syringae* isolated from peach, pear, apple, plum, sour cherry and raspberry. Adoption for a particular host appears to affect the distribution of repetitive sequences, resulting in fingerprints unique to specific strains. Our experiment demonstrates the potential of ERIC-PCR fingerprinting as a diagnostic tool in determining the difference among the *P. syringae* strains from various origin.