TOXIN PRODUCTION BY PSEUDOMONAS SYRINGAE PATHOVAR
ORIGINATING FROM SWEET CHERRY

Iličić Renata¹, Balaž Jelica¹, Vlajić Slobodan¹, Jošić Dragana², Ognjanov Vladislav¹

¹University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia.
²Institute of Soil Science, Teodora Drazjera 7, 11000 Belgrade, Serbia.

Abstract
Pseudomonas syringae pathovars syringae and morsprunorum race 1, causal agents of sweet cherry die back were investigated for their toxin production. Total of 155 strains isolated from diseases sweet cherries from several location in Vojvodina Province, Serbia were used. In previous study the strains were identified as a pv. syringae(79 strains, based on presence/absence of syrB and syrD genes) and as a pv. morsprunorum race 1 (76, presence cfl gene) based on molecular identification. In this study, bioassay for syringomycin production showed that 64 strains among pv. syringae produced toxin, and 15 have not in the presence of syringomycin sensitive organisms G. candidum, S. cerevisiae and R. pilimanae. However, using bioassay for coronatine production on the potato slices only few strains out of 76 pv. morsprunorum race 1 strains produced coronatine.

Introduction
Bacterial canker caused by P. syringae is one of the most serious diseases of stone fruit trees. Diseases of fruits trees caused by pathovars syringae and morsprunorum result in significant economical losses especially in the past few years in the growing of sweet cherry. The disease usually manifestsin the form of drying buds, shoots and branches, followed by the cancers formation. Barkon infected trees and branches has darkly reddish color, sags and crack. Cankers and necrosis can be associated with orange-brown gummosis[4]. Identification of P. syringae pathovars is based on classical bacteriological test LOPAT[11], GATTAtests[10], pathogenicity tests, additional biochemical tests and various molecular techniques of PCR [2; 15; 3; 14; 8; 6; 7]. Pseudomonas syringae pathovars produces several well-characterized phytotoxic compounds. According to literature P. s. pv. syringae produces a toxin of the lipopeptisipedigpeptide group syringomycin and pv. morsprunorum race 1 toxin coronatine [13; 3; 9]. Both toxins have been implicated as virulence factors in the diseases induced by these bacteria. The ability to produce syringomycin examines in the use of indicator fungi Geotrichum candidum[10]. Also can be applied and Rhodotorula pilimanae and Saccharomyces cerevisiae. Völksch et al. (1989) reported another bioassay on the potato slices for the detection of the presence of toxins (coronatine) specific for P. s. pv. morsprunorum (race 1). Genes responsible for syringomycin synthesis (syrB) and syringomycin secretion (syrD) are specific to the pv. syringae, whereas coronatine production gene (cfl) is specific to the P. s. pv. morsprunorum race 1 [2;15; 3; 7]. The aim of this study was to identify P. syringae pvs. strains originating from sweet cherry by testing their toxin production using bioassays.

Experimental
Strains of pv. syringae (79 strains) and pv. morsprunorum race 1 (76 strains) were grownon NSA (Nutrient-Sucrose-Agar) for 2 days at 26°C. In previous study the strains were identified as a pv. syringae (79 strains) and as a pv. morsprunorum race 1 (76) based on molecular identification. Syringomycinproduction. Strains were streaked on Potato Dextrose Agar medium (PDA) in the form of the circle and grown for 24 hours at 26°C. Cultures of
syringomycin sensitive organisms *Geotrichum candidum, Saccharomyces cerevisiae* and *Rhodotorula pilimanae* were cultivated also for 2 days at 25°C. The surface of the medium was sprayed with a suspension of the indicator organism spores prepared in sterile distilled water (SDW). After incubation (2 days), clear zones of fungi growth inhibition were observed around bacterial colonies as an indication of syringomycin production. **Coronatine production.** For the coronatine production bioassay on the potato slices were performed. Bacterial suspension were prepared in SDW and applied on potato slices. Visualization of coronatine production was confirmed by hypertrophy of the tissue slices caused by bacteria.

### Results and discussion

Phytotoxins of *P. syringae* pvs. coronatine, syringomycin, syringopeptin are the most studied, and each contributes significantly to bacterial virulence in plants. Some of them appear as a consequence of particular toxin production, such as chlorosis (coronatine, phaseolotoxin, and tabtoxin) or necrosis (syringomycin and syringopeptin) [1]. Coronatine induces stunting and hypertrophy of plant tissue and is important for virulence of the pathovars that produce it [2]. Bioassay for syringomycin production showed that, in the presence of all syringomycin sensitive organisms, 64 strain of *P. syringae* produced toxin, and 15 (T23, T24, T25, T26, T27, T28, T29, KBNS85, KBNS86, KBNS88, KBNS89, KBNS90, KBNS91, KBNS92, KBNS94) have not (Figure 1). Strains of *P. morsprunorum* race 1 in the same test were negative. According to our previous study using multiplex-PCR method by implementation of both genes *syrB* and *syrD*, genes were not detected (strains T20, T1, T23, T24, T25, T26, T27, T28, T29). In bioassay strains T23, T24, T25, T26, T27, T28, T29 also were negative, these can be interpreted by the absence or suppression of genes for syringomycin secretion. In strains T20 and T1 presence of syringomycin was detected in the case of all indicators, although genes were not amplified. However, eight strains (KBNS85, KBNS86, KBNS88, KBNS89, KBNS90, KBNS91, KBNS92, KBNS94) in bioassay were also negative, but both genes (*syrB* and *syrD*) were successfully detected using m-PCR [5]. This indicated a higher sensitivity of PCR method in comparison with bioassay test. Variability among isolates originating from the stone fruit species according to bioassay test were reported by other authors [12; 3; 9]. Roos and Hattingh (1983) suggest that the syringomycin production is specific for *P. syringae*, but the 30.2% of tested isolates (intermediate forms) also were positive for syringomycin production. Kaluzna (2011) points out that isolates *P. morsprunorum* (races 1 and 2) reacting negatively, and that most isolates *P. syringae* produces syringomycin, however for individual isolates *P. syringae* reaction is negative, as confirmed by our results. Bioassay on the potato slices for the detection of toxins (coronatine) specific for *P. s. pv. morsprunorum* (race 1) performed in all tested strains (76) were positive for only few strains, which was confirmed by hypertrophy of the tissue slices caused by bacteria. Völkisch et al. (1989) noted variability in this test, which can occur depending on the potato cultivar and the age of tuber tissue. According to obtained results this test is not completely reliable and adequately, because *cfl*- coronatine production gene was successfully detected in the case of all *P. morsprunorum* race 1 strain (76).
Conclusion
In bioassay for syringomycin production 64 stains among pv. syringae produced toxin, and 15 have not. On the potato slices only few strains of pv. morsprunorum (race 1) produced toxin coronatine. Bioassays for toxin production can be useful for P. syringae pathovars differentiation, but only with implementation of some PCR methods. Obtained results indicated a higher sensitivity of PCR method used in previous study in comparison with bioassay test.

Acknowledgements
This work was supported by Serbian Ministry of Education, Science and Technological Development, Project No. III46007 and TR31038.

References