TOXIN PRODUCTION BY *PSEUDOMONAS SYRINGAE* PATHOVARS ORIGINATING FROM SWEET CHERRY

Iličić Renata[1], Balaž Jelica[1], Vlajić Slobodan[1], Jošić Dragana[2], Ognjanov Vladislav[1]
[1]*University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia.*
[2]*Institute of Soil Science, Teodora Drajzera 7, 11000 Belgrade, Serbia.*
e- mail:renatailicic@gmail.com

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 strainsamong 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 usuallymanifestsin the form ofdrying buds, shootsand branches, followed by the cancers formation. Barkon infected treesandbrancheshas darklyreddish 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 lipodepsipeptide 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)[5]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 onpotato 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 consequenceof 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 allsyringomycin sensitive organisms,64 strain of pv. *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 pv. *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 pv. *syringae*, but the 30.2% of tested isolates (intermediate forms) also were positive for syringomycin production. Kaluzna (2011) points out that isolates pv. *morsprunorum* (races 1 and 2) reacting negatively, and that most isolates pv. *syringae* produces syringomycin, however for individual isolates pv. *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 comfirmed by hypertrophy of the tissue slices caused by bacteria. Völksch 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 pv. *morsprunorum* race 1strain (76).

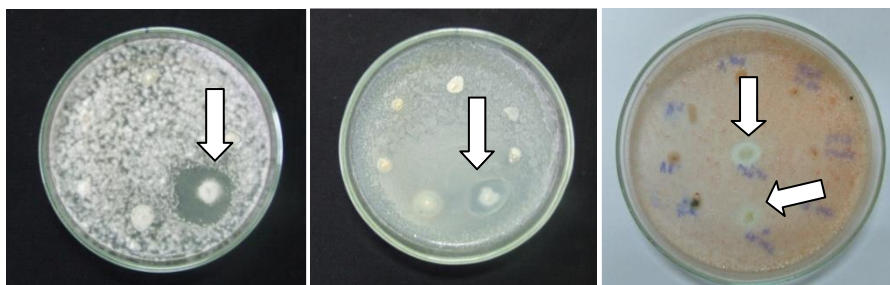

Figure 1. Syringomycin production. Zones of fungi growth inhibition (*G.candidum*; *S.cerevisiae*and *R. pilimanae*)

Conclusion

In bioassay for syringomycin production 64 stains among pv. *syringae*produced toxin, and 15 have not.On the potato slicesonly 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.

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