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# *XANTHOMONAS ARBORICOLA* PV. *PRUNI* ASSOCIATED WITH LEAF SPOT AND TWIG NECROSIS OF PEACH AND SWEET CHERRY IN MONTENEGRO

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## INTRODUCTION

Bacterial spot of stone fruits and almond, caused by *Xanthomonas arboricola* pv. *pruni* (Xap) is one of the most important bacterial diseases of *Prunus* spp. worldwide. The bacterium is listed as a quarantine organism in Montenegro.

In Montenegro, the disease was first described on almond trees in 1998. However, this pathogen was not studied in details since then.

In order to check the status of this pathogen, during the growing season of 2017 and 2018, we carried out survey of stone fruit orchards.

## ISOLATION

From diseased leaves, fruits and twigs bacterial strains were isolated. They formed yellow, convex, and mucoid colonies on yeast extract–dextrose–CaCO3 agar medium (YDC) (Figure 2).

Based on their growth characteristics and preliminary pathogenicity testing (hypersensitivity in tobacco) 37 strains were selected for identification.

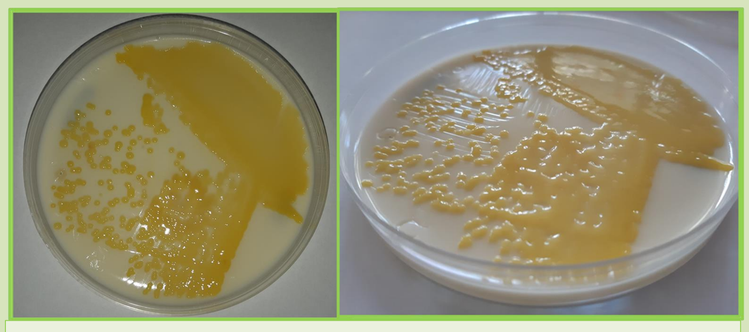

Figure 2. *Xanthomonas arboricola* pv. *pruni*. Development of the colonies on YDC medium (Foto: T. Popović).

## CONVENTIONAL PCR

Specific primers XapY17-F/XapY17-R detecting *ftsX* gene characteristic for Xap strains (Pagani, 2004; Pothier et al., 2011) were used for the identification of strains. As a result of this PCR a product of 943 bp was amplified in all 37 and Xap reference strain (Figure 4).

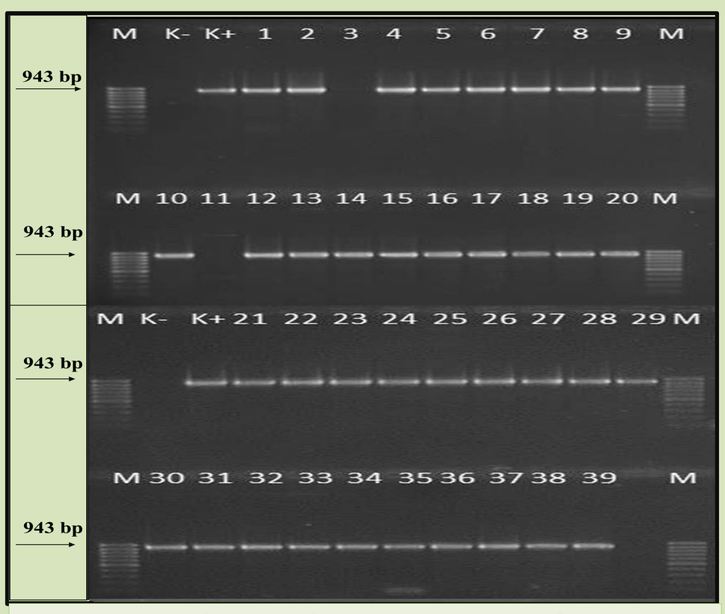


Figure 4. PCR detection ftsX gene in 37 tested strains. K – negative control, K + positive control Xap (69VR - CFBP3892), M- molecular marker (MassRuler Low Range DNA Ladder, Fermentas, Litvanija).


Figure 1. *Xanthomonas arboricola* pv. *pruni*: defoliation on *Prunus persica* (a), leaf and fruit spot on *Prunus persica* (b, c, d), twig cankers on *Prunus avium* (e, f) (Foto: T. Popović).

## CONCLUSION

**According to the results, the strains isolated from peach and sweet cherry in Montenegro were identified as *Xanthomonas arboricola* pv. *pruni*. These plants were detected for the first time as hosts of this pathogen in the country. Therefore, strict phytosanitary measures have to be implemented to control this risk and prevent either import or spread of the pathogen in other areas and other susceptible hosts.**

## SYMPTOMS

During the 2-year survey of stone fruit orchards in Montenegro, peach leaf and fruit spot and twig necrosis were observed near Podgorica, while sweet cherry twig cankers were observed on trees near Ulcinj.

The leaf lesions were initially small, angular, water-soaked, surrounded by a halo. As the disease progressed, the necrotic areas dropped out, leaving a ‘shot-hole’ leaf appearance. Eventually, infected leaves turned yellow and dropped off. On fruits, small, circular, water-soaked or dark brown spots were observed. Cankers on young twigs were dark, elongated, sunken, and accompanied by gummosis. (Figure 1).

## BIOCHEMICAL TESTS

All selected strains were HR+, gram negative, strictly aerobic, oxidase negative, catalase positive, hydrolyzed esculin, and did not grow at 37°C. Based on the biochemical tests, the strains possess characteristics similar to Xap, in spite of the variation in some phenotypic properties.

## PATHOGENICITY TESTS

Pathogenicity was tested in host plants by spraying young shoots and infiltrating leaves and fruits with bacterial suspension ($10^7$ CFU/ml in SDW) of all 37 strains and Xap reference strains. Lesions appeared on all inoculated shoots, leaves and fruits within a week after inoculation (Figure 3).

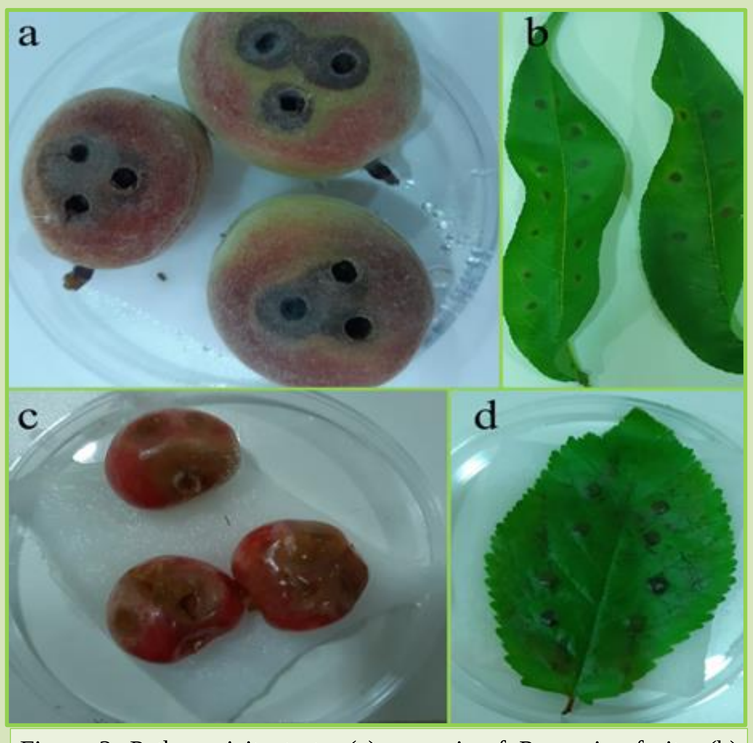


Figure 3. Pathogenicity tests: (a) necrosis of *P. persica* fruits; (b) necrotic spots on *P. persica* leaves; (c) necrosis of fruits of *P. avium*; (d) necrotic spots on *P. avium* leaf (Foto: T. Popović).

## SEQUENCING OF *gyrB* GENE

Amplification and sequencing of *gyrB* gene of ten selected strains was performed using primers described by Parkinson et al. (Parkinson, N., et al. 2007). Obtained partial DNA sequences showed that eight strains share 98.97 to 99.71% of *gyrB* sequence identity with Xap pathotype strain ICMP51. The sequences of two strains showed 100% identity to *gyrB* gene of Xap strains isolated from peach and apricot in Hungary and peach in Italy.

**Acknowledgments**: This work was supported by Administration of Food Safety, Veterinary and Phytosanitary Affairs, Podgorica, Montenegro; and by the Ministry of Education, Science and Technological Development, Republic of Serbia and Faculty of Agriculture contract No. 451-03-68/2020-14/200116, as well as COST Action CA16107 EuroXanth.