Diseases Caused by Bacteria and Phytoplasmas

First Report of *Xanthomonas campestris* pv. *campestris* Causing Marginal Leaf Necrosis of Arugula (*Eruca vesicaria* subsp. *sativa*) in Serbia

A. Prokić, T. Marković, J. Menković, M. Ivanović, and A. Obradović[†]

University of Belgrade, Faculty of Agriculture, Belgrade, Serbia

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Arugula (*Eruca vesicaria* subsp. *sativa* [Miller] Thell., syn. *Brassica eruca* L.) is an annual cruciferous crop that is increasingly grown for fresh consumption in Serbia. In November 2018, a few detached leaves of cultivated arugula originating from a local producer, showing necrotic lesions, were observed in a fresh vegetable market in Belgrade, Serbia. Information about the disease incidence and severity was not available. Intensity of the observed symptoms was low, but it could be a consequence of the produce quality selection for the market. The leaves developed irregular chlorotic lesions starting from the leaf edge, and tissue within some of them turned dark brown and necrotic. From the lesions on different leaves, smooth, bright yellow pigmented, round and opalescent bacterial colonies were isolated on nutrient agar medium after 72 h of incubation at 26°C. Six bacterial isolates, obtained from three leaf subsamples that induced hypersensitive reaction in tobacco leaves (*Nicotiana tabacum* L. cv. Samsun), were selected for further studies. On yeast–dextrose–CaCO₃ medium, the strains formed characteristic creamy yellow, mucoid, opaque, and convex colonies. All isolates were gram-negative, strictly aerobic, nonfluorescent, and catalase positive, did not produce oxidase nor arginine dihydrolase, and did not show pectinolytic activity on potato tuber slices. They hydrolyzed starch, gelatin, and esculin, used glucose and sucrose but not arabinose as a carbon source, and did not reduce nitrates. They grew at 36°C and tolerated 5% NaCl and 0.02% triphenyl-tetrazolium chloride (Lelliott and Stead 1987). These growth characteristics were similar as for the reference *Xanthomonas campestris* pv. *campestris* (*Xcc*) strain KFB 105, used in all tests as a positive control (Obradović et al. 2000). The isolates were further characterized by PCR using primers DLH120/DLH125, specific for the *hrpF* gene region of *X. campestris* according to Berg et al. (2005). A specific DNA fragment of 619 bp was amplified for all tested isolates. Amplification and partial sequencing of the *gyrB* gene of four isolates were performed using set of primers described by Parkinson et al. (2007). All obtained partial *gyrB* sequences

were identical to each other. According to BLAST analysis (GenBank nos. MW508894 to MW508897) they shared 100% of sequence identity with different *Xcc* strains and 99.5% with the *X. campestris* pv. *raphani* pathotype strain deposited in the NCBI GenBank database. Pathogenicity of the isolates was tested by spraying leaves of 3-week-old *E. vesicaria* subsp. *sativa* seedlings grown in a commercial potting mix in a greenhouse, with a 24-h-old bacterial culture suspended in sterile distilled water (10⁷ CFU/ml). *Xcc* strain KFB 105 was used as a positive and sterile distilled water as a negative control. Inoculated plants were incubated under plastic bags for 48 h and further maintained in a greenhouse at ~28°C. On inoculated plants, chlorotic lesions, spreading from the leaf margins, further coalescing into irregular, V-shaped tissue necrosis associated with blackening of veins, developed up to 2 weeks after inoculation. The colonies reisolated from symptomatic leaves were identified using PCR, as described above. Based on studied characteristics, all six isolates associated with arugula leaf lesions in Serbia belong to a clonal population. They were identified as *X. campestris* pv. *campestris*, the causal agent of black rot, a major disease affecting crucifers, including arugula, worldwide (Romero et al. 2008; Rosenthal et al. 2018). So far, it has been described on *Brassica oleracea* and *B. napus* in Serbia (Obradović et al. 2000; Popović et al. 2019). This is the first report of *Xcc* infecting arugula in this country. The severity of the symptoms developed on artificially inoculated plants indicated significant potential of the pathogen to affect the arugula crop in conditions favoring infection. Being a minor crop, accurate information about severity of arugula diseases in Serbia is not available. Lack of crop rotation and close proximity of other *Xcc* host species on a farm could contribute to further spreading of this problem. Follow-up of this arugula disease should reveal the distribution, population structure, and genetic diversity of *Xcc* strains affecting this crop in Serbia.

References:

- Berg, T., et al. 2005. Plant Pathol. 54:416.
- Lelliott, R. A., and Stead, D. E. 1987. Methods for the Diagnosis of Bacterial Diseases of Plants. Blackwell Scientific Publications, Oxford U.K.
- Obradović, A., et al. 2000. Plant Prot. 51:255.
- Parkinson, N., et al. 2007. Int. J. Syst. Evol. Microbiol. 57:2881.
- Popović, T., et al. 2019. Plant Pathol. 68:1448.
- Romero, A. M., et al. 2008. Plant Dis. 92:980.
- Rosenthal, E. R., et al. 2018. Plant Dis. 102:1025.

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[†]Indicates the corresponding author.
A. Obradović; aleksao@agrif.bg.ac.rs