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ANTAGONISTIC ACTIVITY OF *BACILLUS* AND *PSEUDOMONAS* SOIL ISOLATES AGAINST *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS*

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Agricultural production was intensified over the past few decades and became dependent on agrochemicals. Using pesticides is a relatively reliable method of crop protection. However, the pathogenic microorganisms affecting plant health still pose a significant threat to food production and ecosystem stability worldwide. The frequent use of chemicals causes some negative effects, such as development of pathogen resistance to the applied agents and their non-target environmental impacts. The demands for pesticide-free food have led to search for its substitutes. Biological control was reported as alternative or/and supplemental way of reducing the use of chemicals in agriculture. Plant growth-promoting bacteria (PGPB) are known as very effective in biological control.

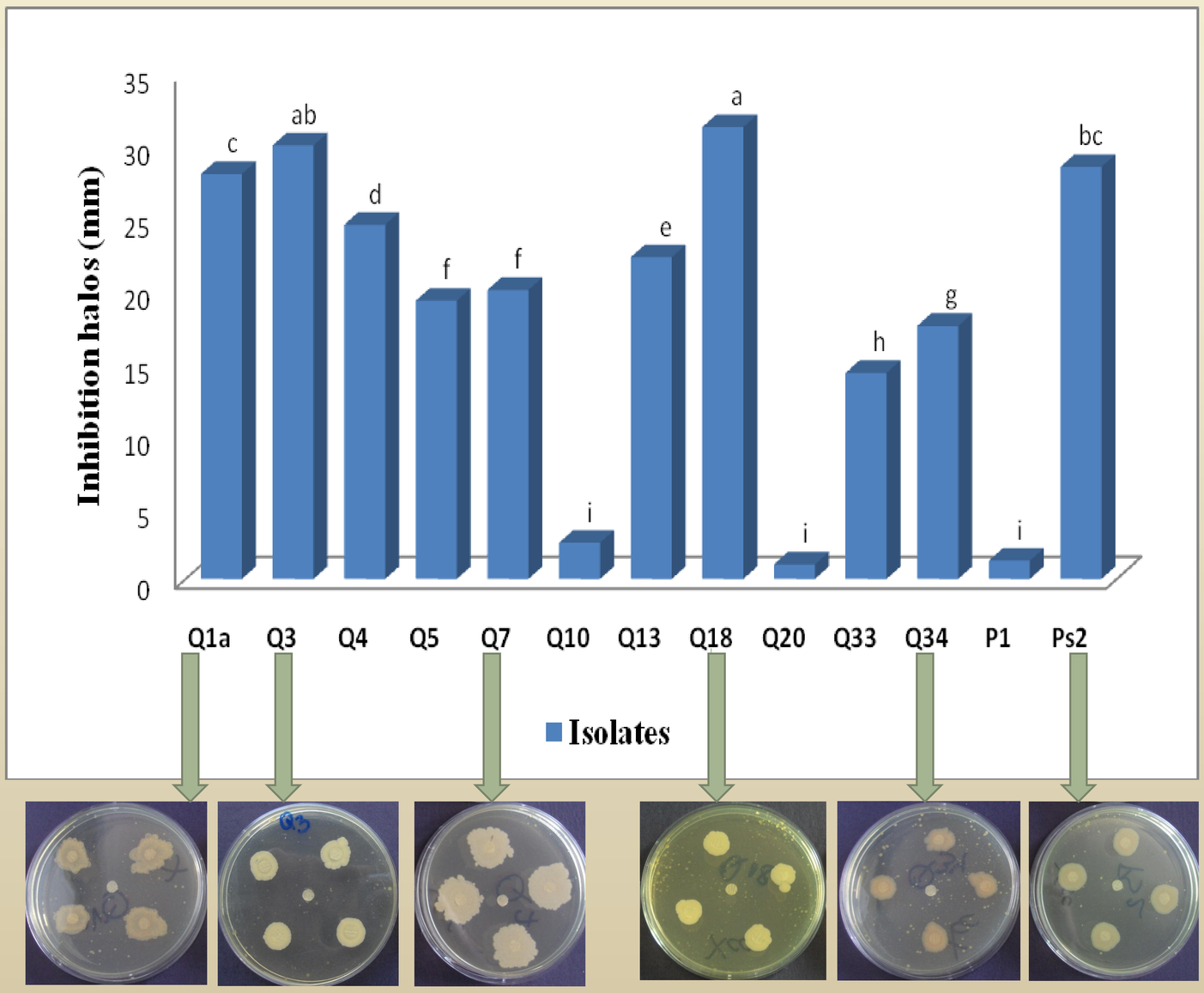
Xanthomonas campestris pv. *campestris* (Pammel 1895) Dowson 1939 (Xcc), causal agent of crucifers black rot, is considered the most serious disease of crucifers worldwide. The most important host is *Brassica oleracea* (including cabbage, cauliflower, broccoli and kale) susceptible to this disease in all developmental stages. Xcc occurs more frequently in warm and humid environments, which are common in tropical and subtropical regions. The symptoms are characterized by yellow V-shaped lesions starting from leaf margins and progressing to the centre through the vascular tissue, resulting, in general, in the leaf necrosis .

The aim of the presented work was to detect antagonistic activities of *Bacillus* and *Pseudomonas* bacteria from the rhizospheric soil against Xcc.

Among a large numbers of isolates from the rhizosfere of different plants, we chose two *Bacillus* (Q3 and Q5) and two *Pseudomonas* (Q4 and Q20) isolates from maize rhizosphere in Sumadija and one *Pseudomonas* from Vojvodina (PS2), two *Bacillus* (Q7 and Q13) and *Pseudomonas* Q33 from pepper rhizosphere, *Bacillus* Q10 and *Pseudomonas* Q1a from alfalfa rhizosphere and from red clover rhizosphere *Bacillus* Q18 and *Pseudomonas* Q34. P1 from oil polluted soil was used as non-rhizospheric *Pseudomonas* isolate. Reference Xcc strain was from National Collection of Plant Pathogenic Bacteria (NCPPB No. 1144). The strain was grown on Yeast Dextrose Chalk medium (YDC) for 48 h at 28°C. Antimicrobial activity was determined by agar diffusion technique. One hundred microliters of Xcc suspensions (3 x 10^8 cells/mL) were mixed in 100 mL Nutrient Agar (NA) and poured in sterilized Petri plates (90 mm in diameter). Tested *Bacillus* and *Pseudomonas* isolates were grown on Nutrient Broth (NB) and 10µl containing 10^6 CFUmL^{-1} (obtained by OD_{600} measurement and calculation) were placed in Petri plates surface and incubated at 28ºC. There were four replicates for each antagonistic bacterium. After three days of incubation, inhibition halos were measured and antimicrobial activity (mm) was expressed as the difference between the diameter of inhibition zone and the diameter of *Bacillus* and *Pseudomonas* colony. All experiments were performed in a completely randomized design. The results were subjected to analysis of variance (ANOVA) and means were compared by Duncan´s Multiple Range Test (P = 0,05) using the software COSTAT.

Growth inhibition of Xcc strain by Q18 and Q3 of *Bacillus* isolates showed significant value of 31,25 and 30 mm, respectively (FIGURE 1). The maximum inhibition value caused by *Pseudomonas* tested was 28.5 mm for Ps2. A very effective *Pseudomonas* isolate Q1a generated similar halo value, since P1 and Q20 isolates showed a minimal inhibition halo of about 1 mm.

FIGURE 1. Antimicrobial activity of *Bacillus* spp. and *Pseudomonas* spp. against *Xanthomonas campestris* pv. *campestris*



All antagonists tested in this study inhibited the phytopathogenic Xcc strains with a different degree of efficiency. Bacillus isolates Q18 and Q3, as a most effective, can be considered for tests in field conditions. It is well known that the genus *Bacillus* is one of the most commonly used for the biocontrol of plant diseases. One of the main mechanisms of action of these microorganisms in biocontrol of phytopathogens is the production of antimicrobial substances, such as lipopeptides, which exibit hemolytic activity. The Xcc antagonistic property, as an additional PGP trait, made this isolates significant for futher field investigation in biological control of different plant pathogens.

As a variety of microorganisms, PGP *Pseudomonas* also exhibit antagonistic activity, attacking pathogens by excreting cell wall lytic enzymes. One of the mechanisms of biological control is the detoxification and degradation of pathogen virulence factors. Also, some pseudomonads use the quorum-sensing capacity to block pathogen by degrading autoinducer signals and blocking expression of numerous virulence genes. The results of this study showed a selection of rhizospheric isolates *Bacillus* Q18 and Q3 and *Pseudomonas* PS2 and Q1a as effective in growth suppression of Xcc pathogenic strain. A large numbers of autochthonous Xcc isolates from different plants will be included in further analysis.