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

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Plant growth-promoting bacteria (PGPB) are commonly present in many environments and are associated with many plant species. The most widely studied group of PGPB are plant growth-promoting rhizobacteria (PGPR) colonizing the root surfaces and closely adhering rhizosphere. Some of the mechanisms of biocontrol mediated by PGPB are: a competition for an ecological niche or a substrate, a production of inhibitory allelochemicals, an induction of systemic resistance (ISR) in host plants to a broad spectrum of pathogens and/or abiotic stresses.

Bacteria genus *Pseudomonas* and *Bacillus* are widely recognized as PGPR effective in biological control of different phytopathogens (fungi, bacteria and viruses). A variety of antibiotics and inhibitory compounds such as amphisin, 2,4-diacetylphloroglucinol (DAPG), hydrogen cyanide, oomycin A, phenazine, pyoluteorin, pyrrolnitrin, tensin, tropolone, and cyclic lipopeptides produced by *Pseudomonas* and oligomycin A, kanosamine, zwittermicin A, and xanthobaccin are produced by *Bacillus*, *Streptomyces*, and *Stenotrophomonas* spp. and form a basis for a biocontrol mechanism of PGPB. Some of the PGP *Pseudomonas* and *Bacillus* are able to attack pathogens by excreting cell wall hydrolases or are able to detoxify of pathogen virulence factors.

Pseudomonas syringae pv. *syringae* van Hall 1902 (Pss) caused bacterial canker of stone-fruit trees and it is one of the most devastating diseases with a loss of 10-75% in young orchards . Disease symptoms include canker development on the shoots and at the spurs base and its progression upwards accompanied with gum exudation early in the growing season. The pathogen attacks twigs, buds, flowers, leaves and fruits. In the early spring, dark brown sunken lesions appear on twigs beneath the infected spurs. A severe infection of the twigs results in shoot blight and death of the infected branches with gums often appearing from cankered regions on the limbs. Pss is also an important pathogen of beans worldwide, causing bacterial brown spot with associated yield losses of up to 55% in South Africa. Bacterial brown spot was reportedly the most widespread bacterial disease of dry bean in South Africa, occurring in 93% of seed production fields and in 100% of commercial fields. Pss survives on a number of crops and non-crop species, which serve as sources of primary inoculum for an infection. Pss is widespread from the tropical areas to the northern Europe and Canada. Pss strains on bean plants are developing resistance to copper fungicides. No method can provide a complete control against bacterial canker and gummosis of fruit trees.

This work was focused on the possibility of indigenous rhizospheric *Pseudomonas* and *Bacillus* strains, already selected as PGP bacteria, to act as inhibitory agents on phytopathogenic Pss, as a first step in investigating of their potential use for the biological control of plant diseases.

Antagonistic activities of six *Bacillus* and seven *Pseudomonas* soil isolates (TABLE 1) against reference Pss strain CFBP 1582 were tested *in vitro* by an agar-diffusion assay. Pss strain was cultured on Nutrient Agar (NA) for 48 h at 28°C. One hundred microliters of Pss 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). After solidification, 10 µL (containing 10^6 $CFUmL^{-1}$) of *Bacillus* and *Pseudomonas* suspensions, grown during 24 h at 28°C in Nutrient Broth (NB), were placed on the agar surface and incubated for 48 h at 28°C. There were four replicates for each antagonistic bacterium. After incubation, the inhibition halos were measured and antimicrobial activity (mm) was expressed as the difference between the diameter the inhibition zone and the diameter of *Bacillus* and *Pseudomonas* colony.

The experiment was performed in a completely randomized design. The results were subjected to the analysis of variance (ANOVA) and means were compared by Duncan´s Multiple Range Test (P = 0,05) using the software COSTAT. Isolates that produced an inhibition zone of 2 mm or more around the agar disks in the lawn of Pss were regarded as antagonistic.

TABLE 1. Bacterial strains used as antagonistic bacteria and their source of isolation

	Isolates	Isolation source		Isolates	Isolation source
Bacillus	Q3	Maize rhizosphere	Pseudomonas	Q1a	Alfalfa rhizosphere
	Q5	Maize rhizosphere		Q4	Maize rhizosphere
	Q7	Pepper rhizosphere		Q20	Maize rhizosphere
	Q10	Alfalfa rhizosphere		Q33	Pepper rhizosphere
	Q13	Pepper rhizosphere		Q34	Red clover rhizosphere
	Q18	Red clover rhizosphere		P1	Oil polluted soil
				Ps2	Maize rhizosphere

Antagonistic bacterial isolates used in this study were selected in the previous investigation on the basis of their PGP traits. All isolates showed at least two traits that cause plant stimulation and/or plant protection or acquisition with nutritive elements. Results of the antagonistic activities of six *Bacillus* and seven *Pseudomonas* soil isolates against Pss in *in vitro* conditions by an agar-diffusion assay, showed that 12 out of 13 isolates produced inhibition zones on the test plates (FIGURE 1). Isolate Q5 inhibited Pss strain less than 2 mm and it was considered as non-antagonistic isolate.

Rhizospheric *Pseudomonas* isolates tested in the study inhibited the growth of Pss more strongly than tested *Bacillus* isolates. The maximum inhibition of Pss growth was demonstrated by the *Pseudomonas* isolate Q34, than Q1a and Q4. *Pseudomonas* Ps2 and *Bacillus* Q13 generated a similar value of inhibition. However, *Bacillus* Q7 isolate was less effective. Some of *Pseudomonas* isolates were already tested for their PGP traits. Isolates Q4 and Q20 from the maize rhizosphere in Sumadija produce HCN, have protease and phospholipase activity, phosphosolubilization ability and isolate Q4 produce a siderophore. Isolate PS2 from maize rhizosphere in Vojvodina is a good producer of lytic enzymes and phenazines and could inhibit growth of many phytopatogenic fungi. *Bacillus* isolates Q7, Q10 and Q13 showed better antagonism to Psp than isolate Q3.

The mechanisms involved in the inhibition of Pss by the most effective *Bacillus* and *Pseudomonas* rhizospheric isolates will be considered in the next step of our investigation.

FIGURE 1 - Antimicrobial activity of *Bacillus* spp. and *Pseudomonas* spp. against *Pseudomonas syringae* pv. *syringae*