

Fungal Inhibition of Plant Growth Promoting Rhizobacteria (PGPR) & Alfalfa Endophytic Bacteria Against *Colletotrichum Trifolii* & *Phytophthora Megasperma*

Maria Valeria Moreno¹, Valeria Arolfo¹, Jose López², Sofia Endorzain², Ezequiel Bigatton³, Ibrahim Ayoub³, Enrique Lucini³, Antonio Lagares², Ariel Odorizzi¹, Daniel Basigalup¹

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Plant Growth Promoting Rhizobacteria (PGPR) and alfalfa (*Medicago sativa* L.) endophytic bacteria can be used to enhance plant growth and improve plant health in alfalfa breeding programs. PGPR interacts with plant rhizosphere through direct and indirect effects. Seeds have been shown to be colonized endophytically by microorganisms, representing natural carriers of a selected microbial inoculum for young seedlings. PGPR and seed endophytes stimulate different positive biotic interactions such as the inhibition of different phytopathogenic fungi by producing antifungal compounds, toxins, or hydrolytic enzymes. In Argentina, alfalfa is mainly affected by diseases caused by *Colletotrichum trifolii* (Ct) and *Phytophthora megasperma* (Pm). It is desirable to control these pathogens through sustainable alternatives. Our objective was to evaluate *in-vitro* fungal inhibition of PGPR and alfalfa endophytic bacteria against Ct and Pm phytopathogens.

Fourteen identified and characterized bacterial isolates were evaluated, ten were rhizospheric (PSE7, PSE, RI1, RI9, SC1, SC2, SC6, MN1, SI2, EZE) and four were endophytic (B2, B4, B12 y B22). Isolates were incubated at 28°C for 48 h to reach 10⁸ CFU.ml⁻¹. Phytopathogens (Ct and Pm) were grown in PDA at 23°C for 15 days. A fungal disc was placed in the center of a PDA plate and two bacterial stretches were made on the sides. The control was a PDA plate with phytopathogen only. Plates were incubated at 28°C for 15 days and experiments were triplicated. Inhibition percentage (IP) was calculated by Rojas-Badía et al. (2017) and variance analysis was performed by Tukey's test. Four isolates (28.6%) did not show inhibition for one or both phytopathogens, while the remaining (71.4%) had mean IP values between 42.5-91.4 for Ct and 61.7-77.3 for Pm at 15 days (Table 1).

Table 1. Mean IP values for Ct and Pm.

Isolate	RI1	RI9	SC1	SC2	PSE	SC6	EZE	MN1	B22	B2	B4
Ct	42.5 ^a	43.2 ^a	46.9 ^a	48.8 ^a	50.8 ^a	52.8 ^a	55.4 ^a	58.4 ^a	80.0 ^b	91.4 ^b	91.4 ^b
Pm	73.1 ^a	74.2 ^a	70.8 ^a	70.9 ^a	0.0 ^b	73.1 ^a	68.4 ^a	70.7 ^a	61.7 ^a	77.3 ^a	77.3 ^a

Different letters indicate statistically significant differences ($p \leq 0.05$).

The statistically significant differences between B2, B4 and B22 with the rest in the Ct experiment could be explained since they are endophytic bacteria isolated from alfalfa seeds and so might show phytopathogenic specificity derived from evolutionary development. Isolates with mean IP values ≥ 50 are candidates for fungal inhibition. Most of the isolates used in this work were from the genus *Bacillus* (86%) that produce various metabolites such as antibiotics, lipopeptides or specific lytic enzymes for phytopathogens. The differences between IP values for both phytopathogens suggest the production of metabolites with different specificity. Four bacterial isolates (B2, B4, SC6 and MN1) were selected to evaluate fungal inhibition against both phytopathogens in a culture chamber and greenhouse.

References

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