



MICROBIOLOGICAL IMAGE

**Antagonistic activity of a *Bacillus* sp. strain isolated in Córdoba, Argentina against *Macrophomina phaseolina* (Tassi) Goid**



**Actividad antagónica de una cepa de *Bacillus* sp. aislada en Córdoba, Argentina contra *Macrophomina phaseolina* (Tassi) Goid**

Verónica Felipe<sup>a,c</sup>, Leopoldo Palma<sup>a,b,c</sup>, Pablo Yaryura<sup>a,b,\*</sup>

<sup>a</sup> Instituto Académico Pedagógico de Ciencias Básicas y Aplicadas (IAPCByA), Universidad Nacional de Villa María (UNVM), Villa María, Argentina

<sup>b</sup> Centro de Investigaciones y Transferencia de Villa María (CIT VM-CONICET), Universidad Nacional de Villa María, Villa María (UNVM), Córdoba, Argentina

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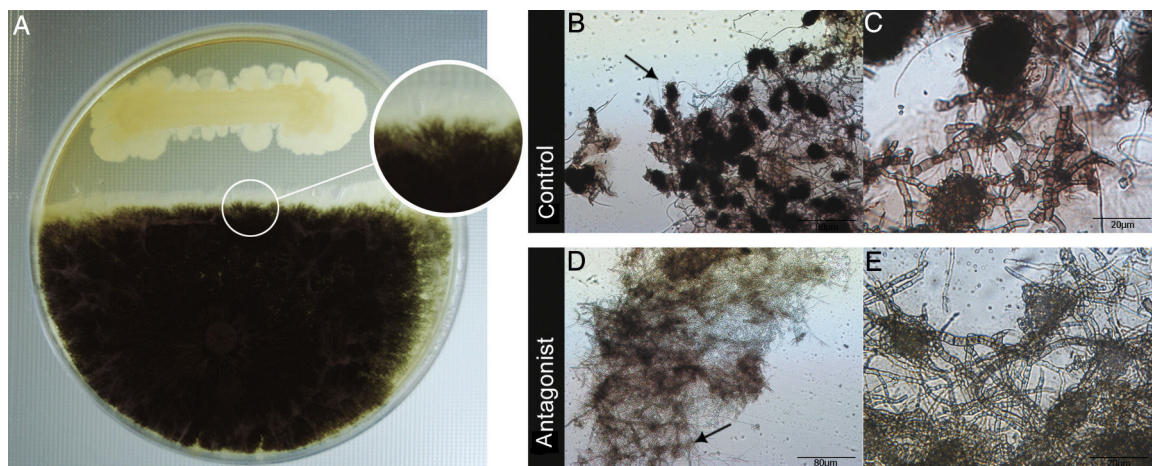
The antifungal activity of *Bacillus* spp. strains has already been reported against a different number of phytopathogenic fungi<sup>3</sup>. Some species belonging to this genus such as *Bacillus subtilis*, *Bacillus pumilus* and *Bacillus licheniformis* are considered GRAS (generally regarded as safe) microorganisms and exhibit potential to enhance plant growth by the production of an ample number of beneficial substances<sup>1</sup>, portraying these species as an environmentally friendly alternative to chemical fungicides. *Macrophomina phaseolina* (Tassi) Goid is a wide range soil-borne phytopathogenic fungus causing charcoal rot on several significant plant crops in Argentina (soybean, common bean, peanuts and corn)<sup>5,6</sup> and is also capable of affecting more

than five hundred plant species worldwide<sup>4</sup>. Its ability to infect these host plants is commonly correlated with the production of viable sclerotia, non-sporic resistance structures constituted by a mass of densely packed hyphae in the field<sup>5</sup>. In this work, we report the isolation of a *Bacillus* sp. strain 11.1 obtained from soil samples from Córdoba Province, Argentina<sup>2</sup> exhibiting a strong antagonistic activity against *M. phaseolina*. In order to analyze the potential antagonistic effect of strain 11.1, both microorganisms were grown on dual culture plates containing potato dextrose agar (PDA) and tryptone soy agar (TSA) (1:1, v/v) and incubated for 48–72 h at 28 °C (Fig. 1). An important and irreversible inhibition of the fungal growth was shown (Fig. 1A). In addition, the fungal mycelium located at the edge of the inhibited region was analyzed by microscopic examination. The most evident antagonistic effect on the fungus was depicted by the lack of black pigmentation and a decrease of sclerotial size in comparison with the control (Figs. 1B and C).

\* Corresponding author.

E-mail address: yaryura@agro.uba.ar (P. Yaryura).

<sup>c</sup> These authors contributed equally to this work.



**Figure 1** Antagonistic activity by *Bacillus* sp. strain 11.1 against fungal pathogen *M. phaseolina* in dual culture assay on PDA/TSA (A). Normal sclerotia formed by *M. phaseolina* (400 $\times$ ) (B) and (1000 $\times$ ) (C). Abnormal sclerotia lacking black pigmentation and smaller size (black arrows) in comparison with the control (400 $\times$ ) (D) and (1000 $\times$ ) (E). Images of the antagonistic assay were obtained by microscopy using an inverted Nikon microscope (Eclipse TI-S) with NIS-Elements software.

## Ethical disclosures

**Protection of human and animal subjects.** The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

**Confidentiality of data.** The authors declare that no patient data are included in this article.

**Right to privacy and informed consent.** The authors declare that no patient data are included in this article.

## Conflicts of interest

The authors declare that they have no conflicts of interest.

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## References

1. Kumar P, Dubey RC, Maheshwari DK. *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. *Microbiol Res.* 2012;167:493–9.
2. Palma L. Protocol for the fast isolation and identification of insecticidal *Bacillus thuringiensis* strains from soil. *Bt Res.* 2015;6:1–3.
3. Petatán-Sagahón I, Anducho-Reyes MA, Silva-Rojas HV, Arana-Cuenca A, Tellez-Jurado A, Cárdenas-Álvarez IO, Mercado-Flores Y. Isolation of bacteria with antifungal activity against the phytopathogenic fungi *Stenocarpella maydis* and *Stenocarpella macrospora*. *Int J Mol Sci.* 2011;12:5522–37.
4. Su G, Suh SO, Schneider RW, Russin JS. Host specialization in the charcoal rot fungus, *Macrophomina phaseolina*. *Phytopathology.* 2001;91:120–6.
5. Torres MJ, Brandan CP, Petroselli G, Erra-Balsells R, Audisio MC. Antagonistic effects of *Bacillus subtilis* subsp. *subtilis* and *B. amyloliquefaciens* against *Macrophomina phaseolina*: SEM study of fungal changes and UV-MALDI-TOF MS analysis of their bioactive compounds. *Microbiol Res.* 2016;182:31–9.
6. Yaryura PM, Córdón G, León M, Kerber N, Pucheu N, Lagorio MG, Rubio G, Vivanco J, García A. Assessment of the role of fluorescent root and seed exudates in crop plants. *J Plant Nutr.* 2013;36:811–24.