

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

22 al 25 de octubre del 2024 Centro cultural y Pabellón Argentina de la Universidad Nacional de Córdoba, Córdoba, ARGENTINA.



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GENOMIC CHARACTERIZATION OF Bacillus ISOLATES WITH BIOCONTROL ACTIVITY AGAINST GRAPEVINE PATHOGENS

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Botrytis cinerea is a major necrotrophic fungal pathogen that causes gray mold, impacting economically significant agricultural and horticultural crops worldwide. This disease can result in substantial yield and quality losses during production and storage, with particular impact on grape production for winemaking. Although various synthetic fungicides are available to control Botrytis, their use in pre- or post-harvest conditions is unsustainable due to the frequent emergence of resistant strains and potential adverse effects on the environment and human health. The viticulture industry faces the ongoing challenge of balancing high productivity with quality, safety, environmental sustainability, and worker health. In this context, plant growth-promoting bacteria, such as those from the genus Bacillus, offer a sustainable alternative to chemical pesticides. Bacillus not only enhances plant growth but also acts as a biocontrol agent against plant pathogens through the production of a diverse array of secondary metabolites with antimicrobial properties. In previous work, we characterized bacteria isolated from the rhizosphere of grapevines, identifying five Bacillus strains that effectively inhibit both Botrytis cinerea and Alternaria alternata, the latter being a cause of grape bunch rot. Notably, while some isolates were effective against both pathogens, others exhibited a broader spectrum of antifungal activity. This study aimed to analyze the genome sequences of these isolates, perform an insilico comparison of genes related to antibiosis mechanisms, and establish their taxonomic position through phylogenomic analysis. Genomes were sequenced using Oxford Nanopore Technologies, yielding sizes between 4,094,482 and 5,811,605 base pairs, and the number of coding sequences (CDS) varying from 5,764 to 4,056. Genomic annotation with RAST revealed that these bacteria are metabolically versatile, with a high abundance of genes related to carbohydrate and amino acid metabolism, among other functions. Notably, between 23% and 29% of the genome sequences were well-characterized in existing databases. Furthermore, we identified gene clusters involved in the production of various

secondary metabolites, including non-ribosomal peptides (NRPS), lipopeptides, and ribosomally synthesized and post-translationally modified peptides (RiPPs), using antiSMASH and PRISM. Taxonomic classification proved challenging due to the genetic similarity among *Bacillus* species. However, using tools such as JSpecies, TYGS, and BTyper3, we classified three strains within the *Bacillus cereus* group and two within the *Bacillus subtilis* group. Future work will focus on refining taxonomic classification to confirm species identities and exploring the mechanisms by which these isolates inhibit *Botrytis cinerea* and *Alternaria alternata*. Understanding these mechanisms will enhance biocontrol strategies and reveal broader applications of Bacillus strains in sustainable agriculture.

Palabras clave: Biocontrol - Bacillus - Botrytis - Grapevine