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Antifungal activity of *Bacillus velezensis*: integrating Volatile Organic Compounds and genomic insights

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Plant growth-promoting bacteria (PGPB), particularly species of *Bacillus*, have emerged as sustainable alternatives to agrochemicals, functioning as biofertilizers and biocontrol agents. Among them, *B. velezensis* stands out for producing antimicrobial metabolites and volatile organic compounds (VOCs), although the identity and roles of these VOCs in specific strains remain poorly understood. This study investigated the antagonistic activity of *B. velezensis* LGMB12 against agricultural phytopathogens, focusing on VOC production and genetic determinants. In vitro assays were conducted in Petri dishes with and without a physical barrier, combined with VOC characterization by HS-GC-MS and comparative genomics with strains LGMB319, LGMB426 and FZB42. The phytopathogens tested were *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Penicillium digitatum*, *Penicillium italicum* and *Monilinia fructicola*. In assays without a barrier, strong fungal inhibition was observed, reaching 99.3% for *S. sclerotiorum* and 98.34% for *M. fructicola*. In barrier assays, fungal mycelial whitening was observed in all treated plates, indicating physiological stress. Notably, *M. fructicola* showed increased growth ($p = 0.00024$) in the presence of VOCs, suggesting a hormetic response. VOCs produced by LGMB12 included ketones (2-nonadecanone, 6,10,14-trimethyl-2-pentadecanone, and 6,10-dimethyl-2-undecanone) and fatty acids (n-pentadecanoic and n-octadecanoic acids), compounds associated with antifungal activity, some of which have limited reports in the literature. Genes such as *acuA*, *acuC*, and *bdhA* were investigated as putatively involved in their biosynthesis. Genomic analysis targeted antagonistic operons (*bac*, *srf*, *pps*, *fenF*), revealing non-synonymous mutations in conserved domains, especially in *pps* and *srf*. Structural predictions using AlphaFold indicated that mutations (e.g., E171D and K123Q in *bacA*, M194R in *bacD*) are located in exposed regions, potentially affecting molecular interactions and antibiotic functionality. These findings highlight the multifunctional potential of LGMB12 as a biocontrol agent acting via direct contact and VOCs, supporting the development of more effective bioinputs for sustainable agriculture.

KEYWORDS: Plant growth-promoting bacteria, Biocontrol, Sustainable agriculture, Plant pathogens

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