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DECODING THE *PENICILLIUM ITALICUM*-CITRUS INTERACTION: FUNCTIONAL INSIGHTS INTO NRPS-DERIVED CYCLIC PEPTIDES IN CITRUS INFECTION

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Penicillium italicum is a postharvest pathogen responsible for the blue mold disease in citrus fruits, particularly under cold and dry storage conditions[1]. Despite its relevance, the molecular determinants of *P. italicum* virulence remain poorly understood[2]. In this study, we uncover a direct link between the fungus's secondary metabolism and its pathogenicity by integrating untargeted metabolomics, gene deletion, and functional assays. LC-HRMS analysis of infected citrus tissues revealed the active and stage-specific production of cyclic peptides, including fungisporin and structurally related tetrapeptides, during host colonization. These compounds are functionally characterized for the first time as secondary metabolites of *P. italicum*. Genome mining identified the *hcpA* gene (PITC_041250) as a candidate NRPS responsible for their biosynthesis. Using the wild-type strain PHI-1 (access number: GCA_002116305.1), we constructed a Δ*hcpA* mutant through homologous recombination by *Agrobacterium tumefaciens*-mediated transformation. Deletion of *hcpA* abolished production of the identified peptides, confirming their biosynthetic origin. Phenotypic analysis showed no defects in growth or sporulation under standard conditions, but the mutant displayed increased sensitivity to osmotic stress. Pathogenicity assays on citrus fruits demonstrated significantly smaller lesions and reduced infection incidence compared to the wild-type, indicating that *hcpA*-dependent peptides are critical for full virulence. This work provides the first functional evidence linking a biosynthetic gene cluster to virulence in *P. italicum*, expanding current knowledge of its secondary metabolism. The findings highlight cyclic tetrapeptides as previously unrecognized virulence factors and potential molecular targets for innovative postharvest control strategies in citrus production. The authors acknowledge the FAPESP: 2022/03594-8, 2023/03831-2, 2022/02992-0. Work at IATA-CSIC was funded by PID2021-126005OBI00, AGROALNEXT/2022/028, PIE 202270I070, and the Severo Ochoa Center of Excellence program CEX2021-001189-S.

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