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Unraveling the Chemical Ecology between Fusarium sp. and Citrus sinensis: Integrating Genomics, Metabolomics, and Biological Assays

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Brazil, the world's largest producer and exporter of oranges, faces major challenges in its citrus industry due to diseases caused by microorganisms[1]. *Fusarium* species, known for producing mycotoxins harmful to human, animal, and plant health, represent a major threat[2]. Recently, we isolated a species responsible for causing irreversible damage to the citrus industry in the Northeast. However, both the disease and the pathogen remain unexplored regarding the chemical and ecological aspects of its secondary metabolism and its role in the pathogen-host interaction. In this study, the pathogen was isolated and subjected to molecular identification. To characterize its biosynthetic potential, complete genome sequencing was performed using the Illumina NovaSeq platform and the genome was assembled using Qiagen CLC Genome Workbench 21.0, with gene cluster analysis performed using fungiSMASH 7.0. To investigate the metabolites involved in host-pathogen interactions, a metabolomics workflow based on untargeted mass spectrometry was applied. Two groups were evaluated at different periods (5, 10, and 15 days): in vitro-inoculated *C. sinensis* plants and healthy plants (controls). In parallel, the same experiment was performed to analyze volatile compounds by solid phase microextraction (SPME) and comprehensive two-dimensional chromatography coupled to quadrupole time-of-flight mass spectrometry (GCxGC-TOFMS). Its biological activity was assessed through antifungal assays and *C. sinensis* seed germination tests. Genome sequencing and assembly resulted in 39 contigs with a total size of 37.28 Mb and a GC content of 48.61%. The fungiSMASH analysis identified 35 Biosynthetic Gene Clusters (BGCs), distributed as follows: 34.29% NRPS, 20% Terpene, 14.29% PKS, 5.71% PKS-Terpene, 8.57% PKS-NRPS, 5.71% Isocyanide, 5.71% RiPP, and 5.71% other compounds. Multivariate PCA analysis revealed a clear discrimination between control and infected plants. In the infected group, a higher abundance of plant defense metabolites was observed, such as phenylalanine (*m/z* 166.0865) and the flavonoid nobiletin (*m/z* 403.1388), which has previously been reported as a defense metabolite in citrus. Other compounds were also observed exclusively in infected samples, including peptides, organic acids, and mycotoxins (*m/z* 338.3417) and (*m/z* 374.2331). The analysis of VOCs revealed significant differences in the chemical profile between healthy and inoculated plants. The SPME technique coupled with GC×GC-TOFMS enabled the identification of a wide variety of VOCs, highlighting the class of sesquiterpenes. To evaluate the influence of the metabolite *m/z* 374.2331 (obtained through in vitro extract isolation) on the pathogen-host interaction, we conducted a germination assay to test its phytotoxicity on citrus seeds. The metabolite *m/z* 374.2331 exhibited a moderate phytotoxic effect at a concentration of 500 ppm and high phytotoxicity at a concentration of 1000 ppm on seed germination. Our biological assays suggest that the metabolite *m/z* 374.2331 may play a significant role in the virulence of the new phytopathogen. Moreover, the mycotoxin inhibited the growth of endophytic fungi isolated from *Citrus sinensis*, belonging to the genera *Colletotrichum* and *Diaporthe*, with minimum inhibitory concentrations (MIC) of 6.25 ppm and 12.5 ppm, respectively. Thus, the obtained results elucidate key aspects of the chemical ecology in pathogen-host interactions, providing a molecular foundation for future investigations.

Keywords: *chemical ecology, citrus, phytopathogen, metabolomics, genomic, interaction, Mycotoxin.*

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