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Microbiome-Guided Discovery of Colletotrichum sp. as an Antagonist of Penicillium italicum in Citrus sinensis Using an Untargeted Metabolomic Approach

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Citrus fruits, such as sweet oranges (*Citrus sinensis*), are highly susceptible to postharvest losses caused by fungal pathogens. Green mold (*Penicillium digitatum*) and blue mold (*P. italicum*) are the predominant decay agents, leading to considerable economic impact. In this study, we employed metagenomics to characterize the microbial communities present on orange peels from healthy fruits and from fruits infected with *P. digitatum* strain Pd1 or *P. italicum* strain PHI-1 (4 days post-inoculation), using 16S rRNA and ITS sequencing. Infection by *P. italicum* resulted in a marked reduction of microbial diversity, with most bacterial and fungal ASVs being suppressed. Within the fungal community, the genus *Colletotrichum* exhibited the most pronounced decrease in abundance. Guided by metagenomic profiling, we isolated 93 cultivable microorganisms from orange peels, including a *Colletotrichum* sp. Furthermore, we performed untargeted metabolomic analyses to investigate the chemical interactions between *Colletotrichum* sp. in association with *P. italicum*. PCA analysis showed a clear trend of separation between groups: co-culture and monocultures. The PCA plot revealed that the quality control (QC) samples clustered closely together, clearly separating from the experimental groups. Additionally, the Random Forest analysis identified 15 variables as key contributors to the discrimination between groups. Furthermore, we used the molecular networking tool of the GNPS online platform to facilitate the metabolic dereplication process. The MS/MS spectral data from the cocultures of wild-type *P. italicum*, and *Colletotrichum* sp., along with their respective monocultures, were integrated into the molecular network, resulting in a total of 3,494 nodes. In co-culture, a significant rise in the relative abundance of *P. italicum*-associated metabolites, including deoxybrevianamide E, brevianamide F, and austamide, was detected. Our results indicate that *P. italicum* modifies the citrus microbiota, leading to a reduction in certain taxa, including fungi of the genus *Colletotrichum*.. The isolation of this antagonist, together with metabolomic analyses, revealed an increased abundance of pathogen-derived metabolites as well as the induction of additional compounds during co-cultivation, underscoring the chemical basis of the inhibitory interaction. These findings highlight the chemical basis of fungal interactions and provide insights into potential strategies for postharvest disease control in citrus. ***Keywords:*** *Citrus x sinensis, Microbiota, Colletotrichum, co-culture, untargeted metabolomics*