



FUNGAL CO-CULTIVATION DYNAMICS AND SECONDARY METABOLITE PRODUCTION: A MULTI-PHASE INVESTIGATION OF ANTAGONISTIC INTERACTIONS AMONG SEVEN PLANT PATHOGENIC SPECIES

Mustapha Ngaski Abubakar^{1*}, Paulo Cezar Vieira¹

mngaskyii@usp.br

¹Faculdade de Ciências Farmacêuticas de Ribeirão Preto, USP - Avenida Professor Doutor Zeferino Vaz, s/n - 14040-903 - Ribeirão Preto, SP, Brazil

This study investigated the complex interactions and secondary metabolite production patterns among six economically important plant pathogenic fungi: *Fusarium guttiforme*, *Pestalotiopsis diospyri*, *Colletotrichum horii*, *C. gloeosporioides*, *F. sacchari*, and *F. verticillioides* as well as an endophyte *Neofusicoccum ribis*. A systematic three-phase experimental approach was employed to evaluate fungal interactions in axenic, dual, and triple co-cultivation systems across multiple growth media. Phase I examined axenic cultures and triple permutations of selected species on rice and potato dextrose broth (PDB) media, revealing enhanced chemodiversity and metabolite yields in rice-based systems. Phase II expanded the investigation to include dual co-cultivation systems using rice medium exclusively, based on Phase I optimization results. Phase III, currently in progress, focuses on three species (*P. diospyri*, *C. gloeosporioides*, and *N. ribis*) selected for their demonstrated overexpression and novel metabolite production capabilities. These organisms were cultivated on rice, rice flakes, and modified Czapek-Dox media with alternative carbon sources (glycerol and suspended flakes), employing varied inoculation ratios and strategic temporal introduction of challenger and inducer strains. Antagonistic interactions predominated across all cultivation systems, with α -pyrone polyketides from *P. diospyri* and *N. ribis* governing the competitive dynamics, consistent with phenotypic observations on potato dextrose agar. Notably, four novel metabolites were isolated from axenic *C. gloeosporioides* cultures grown on rice medium. Metabolite identification employed classical and advanced high-performance liquid chromatography methods, while structural elucidation utilized nuclear magnetic resonance spectroscopy, liquid chromatography-mass spectrometry, and high-resolution mass spectrometry. Principal component analysis is being applied for statistical evaluation of Phase III culture permutations. Promising extracts will undergo scale-up production followed by comprehensive antifungal and protease inhibitory activity assessments. These findings contribute to understanding fungal chemical ecology and may inform biotechnological applications for natural product discovery.

Keywords: Fungal pathogens, co-culture, secondary metabolites, culture-media, bioactivity

