

Untargeted metabolomics and chemical studies of the secondary metabolites from *Aspergillus niger* grown in Palladium (II) medium

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Aspergillus niger is a fungal species widely used in biotechnology to produce enzymes and organic acids. Genetic analysis in fungi has revealed several biosynthetic gene clusters (BGCs), responsible for the biosynthesis of secondary metabolites. The expression of these BGCs has been shown to be highly dependent on the culture conditions, often remaining cryptic or expressed at levels too low for product detection. In this study, we explore the potential of Palladium (II), a non-essential metal, to induce stress responses and possibly trigger the production of novel secondary metabolites in *A. niger*. We performed a metabolomic analysis of *A. niger* grown in potato dextrose agar (PDA) medium containing Palladium (II), monitoring metabolite production by UPLC-ESI-QTOF-MS/MS over 7, 14 and 21 days. Concentrations ranging 100 and 300 mg L⁻¹ of Palladium (II) chloride with a negative control were used to provoke behavior changes (presumably epigenetic change) of the fungus. To enhance our analysis, we extracted the metabolites using a series of solvents of increasing polarity: hexane, ethyl acetate, methanol, and water. Software-guided compound annotation, using MS Dial and MS Finder, identified 36 metabolites, including small organic acids, conjugated amides, coumarin derivatives, naphtho-?-pyrones, sphingolipids, and fatty acid derivatives. Preliminary results indicate pronounced differences in the chemical profile at 14 and 21 days, particularly in lipid content, dependent on Palladium concentration. These findings suggest that Palladium (II) influences the metabolism of *A. niger*, with an omics-driven approach offering deeper insights into this process. Subsequent research will focus on a comprehensive statistical analysis to validate and better understand these metabolic changes, followed by the isolation and characterization of the Palladium-induced metabolites to assess their biotechnological potential.

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