Insights into the Lag and Exponential Phases of the Amazonian Aspergillus flavus Strain through Proteomics

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Aspergillus flavus produces yellow spores and is known for secreting toxins, such as aflatoxins, which affect human and animal health. Initially, during the latent phase, this fungus prepares for growth by adjusting its metabolic functions without visible expansion. Subsequently, in the exponential phase, there is rapid cell division and a significant increase in biomass. However, the lack of detailed understanding of how *A. flavus* regulates its protein responses at each phase may limit the effectiveness of control and treatment strategies. Therefore, shotgun proteomics emerges as an interesting approach to obtaining a comprehensive overview of the fungus's behavior during different growth phases and their molecular characteristics. Thus, this study aimed to investigate the proteomic profile of the secretome from the Amazonian strain *A. flavus* during the latent and exponential growth phases. To this, the strain *A. flavus* CFAM367, deposited in the Amazon Fungal Collection at the Leonidas and Maria Deane Institute (ILMD/Fiocruz Amazônia), was reactivated and purified through serial dilution. The fungus was then cultured in Czapek liquid medium for 96 hours (latent phase) and 120 hours (exponential phase) with agitation at 50rpm and 28°C. The obtained secretome was filtered, concentrated to 5mL with Centripep 3kDa, and subjected to protein extraction via acetone precipitation, followed by solubilization with Rapigest 0.1%. Subsequently, 100µg of proteins were reduced with dithiothreitol (20 mM), alkylated with iodoacetamide (66.7 mM), and digested with trypsin. The peptides were desalted, concentrated, resuspended in 0.1% formic acid, and analyzed using the Easy-nLC II system coupled with the LTQ Orbitrap Velos mass spectrometer, configured in data-dependent acquisition (DDA) mode. Data analysis was performed using PatternLab for Proteomics V. As a result, 57 peptides corresponding to 163 proteins (with redundancy) were identified, with 6 proteins containing at least one unique peptide. When comparing the phases, it was observed that 61% of the identified proteins belong to the exponential phase, compared to 39% in the latent phase. Additionally, the Venn diagram revealed 14 proteins present in all conditions, 7 proteins detected exclusively in the latent phase, and 52 proteins specific to the exponential phase. Among the identified proteins, the most abundant in the latent phase is the C2H2-type transcription factor (TFIIIA), which is primarily associated with the regulation of genes involved in environmental adaptation and cellular differentiation, such as reproductive structure formation and transition between different morphological forms. In the exponential phase, the most abundant proteins include alkaline proteases, glycoside hydrolases, and superoxide dismutase, which are involved in protein and carbohydrate degradation, defense against oxidative stress, and cell wall modulation. Therefore, the analysis identified significant differences in protein expression, with adaptation-related proteins in the latent phase and proteins involved in degradation and defense in the exponential phase. These data can help to understand the molecular mechanisms of this fungus and contribute to a more effective approach in treating infections and toxins associated with *Aspergillus flavus.*

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