The Aspergillus flavus secretome during kojic acid production in the presence of glucose.

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Aspergillus flavus is a deuteromycete fungus belonging to the division Ascomycota, class Ascomycetes, order Eurotiales, and family Trichomaceae. It is a filamentous fungus that produces secondary metabolites, such as kojic acid (AK), through fermentation using carbon sources like xylose, glucose, and sucrose. Studies suggest that the direct conversion of glucose to AK leads to the production of this molecule, however few studies have explored the enzymes secreted during this process. Therefore, to identify these enzymes, researchers induced AK production in *A. flavus* by cultivating it with glucose as the carbon source, and the secreted enzymes were identified using proteomic techniques. *A. flavus* IOC 3974, was used in the experiments. conidia were transferred to Petri dishes containing 20 ml of sterile Czapek Dox agar (CDA) at pH 6. The fungi were incubated at 30°C for 5 days, and then removed from the agar containing mycelium and transferred to Erlenmeyer flasks, containing 100 ml of liquid Czapek medium, supplemented with 120 g L-1 of glucose. The Erlenmeyer flasks were agitated at 180 rpm and 30°C for 72 hours. The culture media were filtered and 0.5 g of the mycelium pellet was inoculated and transferred to new Erlenmeyer flasks containing the same previous cultivation conditions from which the fungus was derived (in quadruplicate procedures)9, at 180 rpm and 37°C for 6 days, and the production of kojic acid (AK) was measured daily by spectrophotometry at 265 nm. On the sixth day of cultivation, the culture medium was filtered and secreted proteins were precipitated, digested by trypsin and subjected to analysis by using the nanoElute nanoflow chromatographic system (Bruker Daltonics, Bremen, Germany) coupled online to a hybrid trapped ion mobility spectrometry-quadrupole time-of-flight mass spectrometer (timsTof Pro, Bruker Daltonics). On the six day of cultivation, the production of 6.71 g/L of kojic acid was observed, and 17 proteins were identified. Among the expressed enzymatic classes in *A. flavus*, hydrolases and glycosidases (3.2.1-) were the most abundant. Examples of enzymes from these functional groups include Probable beta-glucosidase A (E.C:3.2.1.21), Probable mannosyl-oligosaccharide alpha-1,2-mannosidase 1B (E.C:3.2.1.113), Probable beta-galactosidase A (E.C:3.2.1.23) and Probable endo-beta-1,4-glucanase D (E.C:3.2.1.4). The protein ‘Probable beta-galactosidase’ (E.C:3.2.1.22) is an enzyme that participates in the catabolic process of polysaccharides (GO:0000272) and are also produced by microorganisms such as *Bacillus circulans* and *A. oryzae*. During the production of AK in the presence of glucose, a comprehensive overview of the enzymes secreted by *A. flavus* was obtained. These proteins are involved in various biological processes, with a focus on carbohydrate metabolism. These proteins are important for the metabolism of *A. flavus* during AK production and are widely used in industry.

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