Secretome analysis of *Aspergillus fumigatus* reveals Asp-hemolysin as a major secreted protein.

Wartenberg D, Lapp K, Jacobsen ID, Dahse HM, Kniemeyer O, Heinekamp T, Brakhage AA (2011) Secretome analysis of *Aspergillus fumigatus* reveals Asp-hemolysin as a major secreted protein. *Int J Med Microbiol* 301(7), 602-611.

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Abstract

Surface-associated and secreted proteins represent primarily exposed components of Aspergillus fumigatus during host infection. Several secreted proteins are known to be involved in defense mechanisms or immune evasion, thus, probably contributing to pathogenicity. Furthermore, several secreted antigens were identified as possible biomarkers for the verification of diseases caused by Aspergillus species. Nevertheless, there is only limited knowledge about the composition of the secretome and about molecular functions of particular proteins. To identify secreted proteins potentially essential for virulence, the core secretome of A. fumigatus grown in minimal medium was determined. Two-dimensional gel electrophoretic separation and subsequent MALDI-TOF-MS/MS analyses resulted in the identification of 64 different proteins. Additionally, secretome analyses of A. fumigatus utilizing elastin, collagen or keratin as main carbon and nitrogen source were performed. Thereby, the alkaline serine protease Alp1 was identified as the most abundant protein and hence presumably represents an important protease during host infection. Interestingly, the Asp-hemolysin (Asp-HS), which belongs to the protein family of

aegerolysins and which was often suggested to be involved in fungal virulence, was present in the secretome under all growth conditions tested. In addition, a second, non-secreted protein with an aegerolysin domain annotated as Asp-hemolysin-like (HS-like) protein can be found to be encoded in the genome of A. fumigatus. Generation and analysis of Asp-HS and HS-like deletion strains revealed no differences in phenotype compared to the corresponding wild-type strain. Furthermore, hemolysis and cytotoxicity was not altered in both single-deletion and double-deletion mutants lacking both aegerolysin genes. All mutant strains showed no attenuation in virulence in a mouse infection model for invasive pulmonary aspergillosis. Overall, this study provides a comprehensive analysis of secreted proteins of A. fumigatus and a detailed characterization of hemolysin mutants.

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