Optimisation of a 2-D gel electrophoresis protocol for the human-pathogenic fungus *Aspergillus fumigatus*.

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Abstract

Aspergillus fumigatus is the most important airborne fungal pathogen causing life-threatening infections in immunosuppressed patients. One of the important questions concerning A. fumigatus is the identification of pathogenicity determinants. To obtain a comprehensive overview about the proteins produced at different physiological conditions that are related to the infectious process a proteomic approach has been applied. Here, 2-D gel electrophoresis for filamentous fungi was optimised concerning removal of interfering compounds, protein extraction and separation methods. A trichloroacetic acid-based precipitation method of proteins with their subsequent solubilisation by the use of a combination of CHAPS with a second sulfobetaine detergent gave the best results. The optimised protocol was evaluated by the analysis of the proteomes of A. fumigatus grown on two different carbon sources, i.e., glucose and ethanol. Carbon catabolite repression has not been studied in detail at the protein level in A. fumigatus yet. In addition, growth on ethanol leads to activation of the glyoxylate cycle which was shown to be essential for pathogenesis in bacteria and fungi. In A. fumigatus, differential patterns of enzymes of the

gluconeogenesis, glyoxylate cycle and ethanol degradation pathway during growth on glucose and ethanol were observed.

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