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Title: Protein identification of two allergens of boletus edulis causing occupational asthma

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Body: BACKGROUND: Boletus edulis, a highly valued edible mushroom, has been implicated in occupational asthma. While Boletus edulis allergens have occasionally been characterized using immunoblotting, to our knowledge their proteins have not been identified to date. The aim of this present study was to further identify these allergens using a proteomics approach. METHODS: A crude extract of Boletus edulis proteins was separated by SDS-PAGE and immunoblotting was then performed with the use of a serum from a patient with occupational asthma to Boletus edulis confirmed by a positive specific inhalation challenge. Protein identification was performed with DeNovo sequencing using PEAKS Studio 5.3 software (Bioinformatics Solutions Inc.). Briefly, the Boletus edulis protein bands separated by one-dimensional SDS-PAGE were excised from the gel and digested with trypsin according to standard protocols. The tryptic peptides were analyzed with a maXis UHR-Qq-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) and subsequently sequenced using both the DeNovo and the Spider Homology Search tools from the PEAKS Studio 5.3 software. RESULTS: Immunoblotting performed with the Boletus edulis powder extract showed two IgE-binding bands at approximately 17 and 55 kDa. The mass spectrometry identified a 16 kDa band as a fungal fruit body lectin and a 46 kDa band as a protein of the glycoside hydrolase family. CONCLUSIONS: Although few cases of OA due to inhalation of Boletus edulis have been described to date, its prevalence may well be increasing due to its wide use in the food industry. The sequencing of the proteins responsible may be a first step forward in the design of future therapeutic strategies.