A Possible New Mushroom Allergen in a Case of Occupational Asthma

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Basidiomycetes is the largest fungal complex. It comprises mushrooms, puffballs, toadstools, and bracket fungi, which are rarely considered allergenic [1,2]. However, a high prevalence of work-related respiratory morbidity has been reported among mushroom farm workers, including upper airway symptoms, asthma, and a specific form of hypersensitivity pneumonitis known as "mushroom worker's lung" [3]. Most reports come from eastern Asia, where mushrooms are extensively cultivated and consumed [4]. However, the increase in exotic mushroom consumption in Europe has led to an expansion of local production, with a higher risk of work-related diseases in various geographic areas [5].

We report the case of a 32-year-old woman from Jaén (Andalusia, southern Spain) who had been residing in Portugal for 7 years. The patient was referred to our Allergy Department for worsening of chronic nasal symptoms and new, recurrent episodes of dyspnea and wheezing (starting 12 months earlier). She had been working on a mushroom farm for 7 years and was involved in the growing and packing of 6 mushroom species. Respiratory symptoms were exacerbated in the workplace, especially inside the greenhouse where the mushrooms were grown. The patient had a personal history of grass pollen allergic rhinitis since childhood, although this was controlled with immunotherapy.

Spirometry at her first visit revealed normal baseline lung function, with a positive bronchodilation test result. Skin prick tests (SPTs) with extracts of common inhalant allergens (LETI) were positive to house dust mite (*Dermatophagoides farinae*), tree pollen (plane tree, birch, and olive tree), grass pollen mix, weed pollen mix, and pellitory. Testing of *Cladosporium herbarum* and *Aspergillus fumigatus* extracts yielded negative results. SPT with nAlt a 1 extract (Diater) was positive (mean diameter, 4 mm; histamine 10 mg/mL, mean diameter, 5 mm).

Serum specific IgE (ImmunoCAP Specific IgE, Phadia AB) was below the detectable level for *C herbarum, A fumigatus*, and *Penicillium notatum*, but elevated for *Alternaria alternata* (3.69 kU/L). These results were confirmed by ImmunoCAP ISAC (Phadia AB) for rAlt a 1 (17.9 ISU-E).

The diagnosis of occupational asthma was confirmed based on serial FEV_1 and PEF monitoring at work (mean FEV_1, 1.47 L [53%]; mean PEF, 201 L/min) and away from work (mean FEV_1, 3.39 L [123%]; mean PEF, 517 L/min) [6].

The patient sampled 6 mushroom species (Supplementary material) from her workplace (*Flammulina velutipes* [enoki], *Agaricus bisporus, Agaricus brunnescens, Lyophyllum shimeji, Lentinula edodes*, and *Pleurotus ostreatus*). SPTs were performed with the cap, stalk, and gills, eliciting positive results to all parts of *F velutipes, A bisporus, A brunnescens, L shimej*i, and *P ostreatus* (all were negative in 6 nonatopic negative controls). Allergen extracts were produced from the sampled mushrooms (10 mg/mL; Diater) for SPTs, which elicited a positive reaction (4 mm) with both *A bisporus* and *A brunnescens*. Samples were used to perform SDS-PAGE followed by immunoblotting, as described elsewhere [7,8]. A high intensity 12-kDa IgE-binding protein was identified from *L shimeji*; 3 other lower intensity bands with molecular weights of 12, 30, and 45 kDa were identified in *F velutipes* (Figure).

The patient was able to tolerate oral intake of all the mushrooms tested and denied any contact symptoms elicited by these species.

We report a case of occupational asthma induced by IgE-mediated sensitization to mushrooms, supported by the identification of IgE-binding proteins in an immunoblotting assay.

L shimeji, F velutipes, A bisporus, and *A brunnescens* seem to be the relevant allergen sources. Although SPT results were positive to *A bisporus* and *A brunnescens*, we were unable to identify any IgE-binding proteins in vitro. Despite the negative SPT results, immunoblotting revealed a high-intensity 12-kDa IgE-binding band from *L shimeji* and 3 fainter bands of about 12, 30, and 45 kDa from *F velutipes*. The extracts used in the SPTs were specially produced for this patient at a concentration that was validated in 6 negative controls, thus potentially explaining the discrepancies found.

Cross-reactivity with *A alternata* does not explain our findings, since the patient seemed to be sensitized exclusively via Alt a 1, which has a higher molecular weight (15-16 kDa

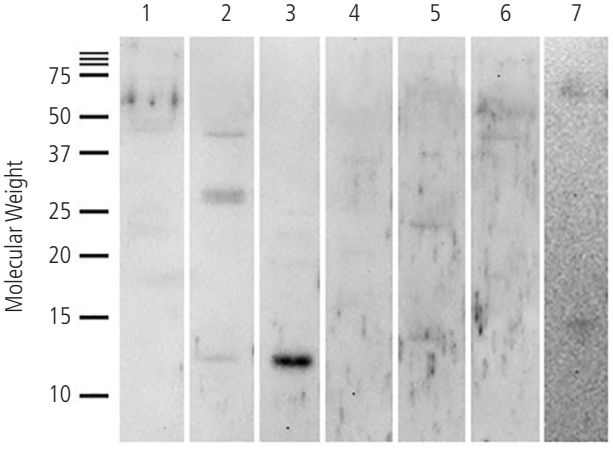

Figure. Immunoblotting results. Lane 1, *Lentinula edodes*. Lane 2, *Flammulina velutipes*. Lane 3, *Lyophylum shimeji*. Lane 4, *Pleurotus ostreatus*. Lane 5, *Agaricus brunnescens*. Lane 6, *Agaricus bisporus*. Lane 7, Alt a 1.

under reducing conditions) than the proteins identified in mushrooms (Figure). Mushrooms are macrofungi with edible bodies that belong to the Basidiomycota phylum, whereas molds such as *A alternata* are classified as Ascomycota. It has been suggested that the cross-reactivity between these 2 classes is minimal [10], consistent with our results.

Allergic reactions to the mushroom species mentioned are extremely rare. *L shimeji* belongs to the species complex *Lyophyllum decastes*, within which taxonomic confusion is frequent, both in the scientific literature and in herbaria [9]. This is probably explained by the micro- and macromorphology of the species included and by the considerable intraspecific plasticity in terms of basidiocarp size and shape, gill attachment to the stem, and coloration [9]. While there are several reports in the literature regarding shimeji-induced respiratory disease in this setting, to our knowledge, none refers specifically to *L shimeji*, but rather to *Tricholoma conglobatum* (also known as *Lyophyllum fumosum*) or *Lyophyllum aggregatum* (Supplementary material).

Two cases of allergic reactions after ingestion of *F velutipes*, with involvement of a 75-kDa IgE-binding protein are shown in the Supplementary material. *A bisporus* has been involved in 2 cases of work-related asthma, with immunoblotting showing 2 intense IgE-binding bands of 15.8 kDa and 13.8/14.5 kDa, as well as several minor bands of 24-39 kDa (see Supplementary material). It has also been reported that 23.6% of mushroom farm workers were sensitized to *A bisporus* (Supplementary material).

In this report, we add *L shimeji, F velutipes*, and, potentially, *A brunnescens* and *A bisporus* as new allergenic sources that could elicit work-related asthma and rhinoconjunctivitis. Furthermore, immunoblotting revealed IgE-reactive proteins in *L shimeji* and *F velutipes* that have not been described in the literature. However, this report is limited by the absence of characterization of specific proteins from fungal spores and parts of the mushroom body. Various allergenic proteins have been described in mushroom spores and basidiocarps and reported to cause IgE-mediated reactions (Supplementary material).

To our knowledge, this is the first report of a case of occupational asthma and rhinoconjunctivitis with suspected involvement of *L shimeji, F velutipes*, and *A brunnescens*, and the third with involvement of *A bisporus*.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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