

Journal Pre-proof

Occupational Respiratory Allergy Caused by Oyster Mushroom (*Pleurotus ostreatus*)

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NO OUTPUT

Clinical Communication

Occupational Respiratory Allergy Caused by Oyster Mushroom (*Pleurotus ostreatus*)

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Running head: Respiratory allergy to oyster mushroom

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Clinical Implications

This report highlights the respiratory sensitizing potential of edible mushrooms and provides further evidence supporting an IgE-mediated mechanism.

Edible basidiomycetes mushrooms are increasingly consumed and cultivated indoor worldwide. A few reports have documented occupational asthma (OA) in mushroom cultivators and processors and in workers exposed to dried mushrooms, including white button mushrooms (*Agaricus bisporus*), porcini (*Boletus edulis*), shiitake mushrooms (*Lentinula edodes*), and oyster mushrooms (*Pleurotus ostreatus*) [1-6], but basidiomycetes allergens causing airway sensitization are still poorly characterized.

We describe a cook who developed OA caused by fresh oyster mushrooms ascertained by a SIC with assessment of changes in markers of airway inflammation, identification of specific IgE antibodies (sIgE), and characterization of a 60-kDa allergen.

A 42-year-old ex-smoker reported a history of allergic asthma and rhinitis since adolescence. His asthma was controlled by treatment with a combination of inhaled fluticasone propionate/salmeterol 500 µg/50 µg once in the morning. He started working as a cook in a catering facility at the age of 18 years. Approximately 5 years after entering this job, he noticed that his asthma and rhinitis symptoms worsened on the days the mushrooms were delivered, cleaned and sliced. He had to take an inhaled short-acting β_2 -agonist up to 10 times on those days. Over the following twenty years, work-related asthma symptoms progressively worsened, predominantly upon exposure to oyster mushrooms. The asthmatic attacks occurred immediately after unloading and carrying boxes filled with mushrooms. The subject experienced five severe asthma exacerbations requiring oral corticosteroids and was admitted twice to the emergency room for an asthma attack after a work shift.

The patient was evaluated 20 months after he had stopped working as a cook due to severe work-related asthma symptoms, and avoided any exposure to fresh mushrooms. He reported neither allergic reactions after eating mushrooms nor skin symptoms related to mushroom contact. Skin-prick tests with a battery of common allergens elicited a positive response to house dust mite, cat dander, and grass pollen. Prick-to-prick tests were strongly positive for oyster mushroom gills and cap and negative for *A. bisporus*.

The subject gave informed consent to complete a SIC with oyster mushrooms (Figure 1). Baseline spirometry showed a forced expiratory volume in 1 second (FEV₁) of 3.39 L (86% predicted) and a FEV₁/forced vital capacity ratio of 78%. As a control challenge, the subject sliced potatoes for 30 minutes without eliciting significant changes in FEV₁ over the following 7 hours. At the end of this control day, bronchoprovocation with histamine using the tidal breath method showed that the concentration of histamine inducing a 20% decline in FEV₁ (PC₂₀) was 0.18 mg/mL, indicative of marked nonspecific bronchial hyperresponsiveness. Induced sputum collected by inhaling increasing concentrations (3%, 4%, and 5%) of nebulized hypertonic saline revealed an eosinophil count of 2.5%.

On the next day, shaking and pouring fresh oyster mushrooms from one container to another for a cumulative duration of 60 min elicited a late asthmatic reaction with a maximum fall in FEV₁ of 27% from baseline value six hours after the end of exposure. Inhaled salbutamol (400 µg) was then administered and sputum was induced. The eosinophil count was markedly increased to 24.5%. Twenty-four hours after the end of challenge exposure, the FEV₁ was still decreased to 74% of the pre-challenge value and sputum eosinophil count was 6.5%. The level of fractional exhaled nitric oxide (FeNO) was increased to 46 ppb compared with the pre-challenge assessment (13 ppb).

For further immunological investigations, extracts of lamellae, cap, and stalk fractions of oyster, white, and shiitake mushrooms were prepared with protein contents ranging from 0.5 to 2.1 mg/mL. The lamellae fraction of the three mushrooms species was biotinylated and coupled to ImmunoCAPs (ThermoFisher, Uppsala, Sweden). An increased level of specific IgE antibodies (sIgE) was detected against oyster mushroom (1.46 kU_A/L), while sIgE levels against white and shiitake mushrooms were less than 0.35 kU_A/L. No sIgE were detected in pooled sera from two unexposed atopic control subjects. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) with Coomassie blue staining showed a comparable protein band pattern in the lamellae, cap, and stalk fractions of oyster mushrooms (Figure 2). Immunoblotting analysis using the subject's serum (Figure 2) revealed strong sIgE

binding to a protein at approximately 60 kDa and a weak reactivity to a 97-kDa protein across the three oyster mushroom fractions. Protein sequencing by tandem mass spectrometry (MS/MS) revealed a highly significant match of the 60-kDa protein covering 46% of the NCBI Reference Sequence XP_036629505.1, annotated as a protein involved in N-glycan maturation, potentially related to α -mannosidase activity.

To our knowledge, this is the first characterization of an allergen involved in the development of respiratory allergy caused by IgE-mediated sensitization to oyster mushroom. The SIC with oyster mushrooms elicited a late asthmatic reaction associated with a marked increase in sputum eosinophils and FeNO, supporting a TH2 inflammatory mechanism. Immunoblotting identified 60-kDa and 97-kDa IgE-binding proteins. A 60-kDa IgE-binding protein has been previously detected in a vegetable market worker with OA due to *P. ostreatus* [6]. In our subject, tandem mass spectrometry identified a 60-kDa IgE-binding protein belonging to the glycoside hydrolase family 47 (GH47) involved in N-glycans processing and quality control of glycoprotein folding. A 46-kDa allergen from the glycoside hydrolase family 5 (GH5) has already been described in *B. edulis* [4], but GH5 and GH47 are functionally and structurally distinct.

Despite the widespread consumption and indoor cultivation of edible basidiomycetes mushrooms, the burden of respiratory allergy among exposed workers remains largely unknown. A questionnaire survey of a mushroom processing plant found a high prevalence (~21%) of work-related respiratory symptoms, although IgE sensitization to mushrooms was not investigated [7]. Tanaka et al. [8] identified cough variant asthma in 24% and eosinophilic bronchitis in 5% of workers employed in a mushroom farm producing *Hypsizygus marmoreus* (Buna-shimeji). On the other hand, the role of exposure to outdoor airborne basidiospores in the development of respiratory allergy remains uncertain [9].

This report highlights the respiratory sensitizing potential of basidiomycetes mushrooms and the need to consider this possibility when evaluating mushroom growers and processors with

110 work-related asthma symptoms. Further characterization of basidiomycetes allergens is
111 essential for the development of more specific diagnostic tests.

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114 manuscript, critical feedback, and final approval of the manuscript.

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LEGEND TO FIGURES**Figure 1.**

Results of specific inhalation challenges illustrating the changes in forced expiratory volume in 1 second (FEV₁) after a control exposure to potatoes for 30 minutes (open circles) and fresh oyster mushrooms (*Pleurotus ostreatus*) for 60 minutes (black closed squares). *Pre*, pre-exposure; *BD*, inhaled bronchodilator.

Figure 2.

Western blot of oyster mushroom (*P. ostreatus*) extracts of lamellae (lane 1, 4, and 7), cap (lane 2, 5, and 8), and stalk (lane 3, 6, and 9): Coomassie staining (lane 1-3) and IgE-binding with the subject's serum (lane 4-6) and pooled control sera (lane 7-9). Molecular weights (MW) of marker proteins are indicated on the left (kDa). A strong IgE-binding band at approximately 60 kDa (arrows) and a faint band at 97 kDa were detected by the subject's serum, but not by the control sera.



