Identification of Ribosomal Proteins as Cross-Reactive Allergens in a Case of Mushroom Food Allergy

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Although a variety of mushroom species are commonly consumed worldwide, mushrooms are a rare cause of IgE-mediated hypersensitivity reactions. One of the common characteristics of mushroom allergy is cross-reactivity among fungal species; however, mushroom allergens are poorly characterized [1–4]. Here, we have presented a Japanese case of immediate-type food allergy caused by four popular mushroom species in which ribosomal proteins were identified as cross-reactive mushroom allergens.

A 21-year-old Japanese man had a 7-year history of recurrent episodes of oral allergic symptoms (oral irritation, throat discomfort, and itching) and cough immediately after consuming meals containing each one of shiitake (*Lentinula edodes*), brown beech (*Hypsizygus marmoreus*), king trumpet (*Pleurotus eryngii*), or hen-of-the-woods (*Grifola frondosa*) mushrooms and the broth of shiitake mushrooms. These symptoms resolved spontaneously within 30–60 minutes of onset. He visited our
hospital for further examination of mushroom allergy. He did not experience any food allergy symptoms after consuming meals without mushrooms. He had a history of asthma and atopic dermatitis from the age of 10–12 years. His total IgE level was 457.0 IU/mL, and the multi-panel IgE test (View Allergy 39®, Thermo Fisher Diagnostics K.K., Tokyo, Japan) revealed positivity for the following allergen-specific IgEs: Japanese cedar (index value: 15.63), Japanese cypress (8.76), Timothy (12.65), orchard grass (16.78), house dust (4.32), Dermatophagoides pteronyssinus (5.50), and shrimp (0.53).

In this test, Alternaria- (0.48) and Aspergillus-specific IgEs (0.33) were detected at the suspected level (index value <0.50). A prick-to-prick test showed a wheal size of 8×7 mm with 10 mg/mL histamine (Torii Pharma, Tokyo, Japan), 1×1 mm with saline, 5×5 mm (2+) with raw Ledodes, 5×5 mm (2+) with broth of Ledodes, 8×9 mm (3+) with raw H. marmoreus, 12×7 mm (3+) with raw Gfrondosa, 10×7 mm (3+) with raw Flammulinavelutipes, and 0×0 mm (-) with raw Auriculariaauricula-judae.

To explore mushroom allergens, 5 g of edible parts of Ledodes, H. marmoreus, Peryngii, and Gfrondosa were minced and homogenized with 1,000 μL of ice-cold phosphate-buffered saline (PBS). After centrifugation at 21,500×g for 10 min, the supernatant of each sample was collected as PBS-soluble protein. SDS-PAGE and immunoblotting were performed, as described previously [5], using 40 μg of each
PBS-soluble protein and 15% polyacrylamide gel. As negative controls, serum from healthy subject and blocking reagent (5% skim milk in Tris-buffered saline containing 0.1% Tween-20) were used as negative controls. Serum IgE antibodies were specifically reacted with a 15-kDa protein for *L. edodes* (Supplementary Figure, A, lane L) and *P. eryngii* (Supplementary Figure, A, lane P). To purify the 15-kDa allergen in PBS-soluble proteins of *L. edodes*, the proteins were fractionated by precipitation with ammonium sulfate and dissolved in PBS as previously described [5]. Immunoblotting of each fraction revealed that the 15-kDa allergen precipitated with 30%–40% ammonium sulfate (Supplementary Figure, B, lane 40).

Next, to identify the 15-kDa allergens of *L. edodes* (Supplementary Figure, B, lane 40) and *P. eryngii* (Supplementary Figure, A, lane P), both protein bands were excised from the Coomassie brilliant blue-stained gel, and the mass spectra of these samples were obtained as previously described [5]. The generated mass lists were searched against the protein databases of *Ledodes*(txid5353) and *Peryngii* (txid5323) from NCBI (access date: 2020.12.19) using the database search software ProteinPilot™ (ver. 4.5; AB SCIEX LLC, Framingham, MA, USA). The 15-kDa allergens of *Ledodes* and *Peryngii* were identified as ribosomal protein S8 (accession no. GAW05875.1) and ribosomal protein S15a (accession no. KAF9498209.1), respectively.
Additionally, we found that the primary structure of these two proteins was significantly similar to 110/127 (87%) amino acid identities using the basic local alignment search tool (BLAST). To evaluate the IgE cross-reactivity between *L edodes* and *P eryngii*, the serum of the patient was pre-incubated with fractionated *L edodes* proteins (Supplementary Figure, B, lane 40; 0, 1, 10 μg) for 2 h at 37°C. As expected, IgE binding to the 15-kDa allergens for *Ledodes* and *Peryngii* was inhibited by preincubation with fractionated *Ledodes* proteins in a concentration-dependent manner (Supplementary Figure, C). These results suggest that ribosomal proteins from *Ledodes* and *Peryngii* mushrooms have cross-reactivity. In this case, we could not determine the cross-reactivity of *H marmoreus* and *G frondosa* because we did not obtain the specific IgE-binding to these proteins in immunoblot analysis. Further considerations are necessary to extract the allergens from these mushrooms.

Allergy to *L edodes* has rarely been reported and it allergens have not been identified. Ito et al. reported that IgE antibodies from a patient with food allergy with three different mushroom species (shiitake, shimeji, and maitake) reacted with the 15-kDa proteins for both raw and boiled *Ledodes* extracts [6]. Pravettoni et al. reported a patient with severe work-related asthma caused by *Ledodes* packaging and confirmed IgE reactivity to *Ledodes* proteins (15 kDa and 24 kDa)[7]. Thus, ribosomal protein S8
(15 kDa) may be a major allergen in *Ledodes*. To our knowledge, *Peryngii* allergy has not been previously reported.

Ribosomal proteins of fungi, such as *Aspergillus fumigatus* and *Alternaria alternata*, are known as mold allergens. These fungi mainly cause respiratory allergic diseases. Food allergies to mycoproteins or button mushroom (*Agaricus bisporus*) due to cross-reaction to mold shaves have been reported[2–4]. In these reports, the patients’ IgE reacted to acidic ribosomal protein P2[2], manganese-dependent superoxide dismutase (MnSOD) [3], orporin family protein [4]. In our patient, a low titer of specific IgE for *Alternaria* and *Aspergillus* was detected in the serum using View Allergy 39®; however, the patient was not sensitized to any mold allergen components contained in ImmunoCAP ISAC™ test (Thermo Fisher Diagnostics), including Asp f 6 (MnSOD from *A. fumigatus*). Furthermore, BLAST analysis revealed that the ribosomal protein S8 (*Ledodes*) and ribosomal protein S15a (*Peryngii*) are not homologous to any reported mold ribosomal protein allergens. In a study by Kayode et al., four patients with mushroom allergy showed positive reactions to multiple mushroom species on the prick-to-prick test; however, only one patient showed positive results for fungal aeroallergens in the skin prick test [1]. The sensitization route should be determined to establish the clinical relevance of mold sensitization and mushroom food allergy and
reveal the pathogenesis of mushroom allergy due to ribosomal proteins.

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**Conflict of interests**

The authors have no conflicts of interest to declare.

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References


## Tables

**Table 1. Amino acid sequence of identified mushroom ribosomal proteins**

<table>
<thead>
<tr>
<th>Origin</th>
<th>Amino acid sequences</th>
</tr>
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<tbody>
<tr>
<td><em>L. edodes</em></td>
<td>iMVRISVLNDC LNNVNAERR GKRQVLVRPS SKVVVKFLSV MQRHYIGEF EIIDDHRAGK</td>
</tr>
<tr>
<td><em>P. eryngii</em></td>
<td>iMVRVSVLND LNNMVNAERR GKRQVLVRPS SKVVVKFLSV MQRHYIGEF EIIDDHRSGK</td>
</tr>
<tr>
<td><em>L. edodes</em></td>
<td>IVIQNLGRNL KTGVISPRFN VQVTQIESIWV NLLLPSSGFG YIILTSGSI LDHEEARRKN</td>
</tr>
<tr>
<td><em>P. eryngii</em></td>
<td>IVQNLGRNL KTGVISPRYN IQANQIESIWV NLLLPARSFG YIILTSGSI MDHEEARRKN</td>
</tr>
<tr>
<td><em>L. edodes</em></td>
<td>VGAMFVAPRR ---------- ---------- ---------- NHDSHSLTAT ELP 493</td>
</tr>
<tr>
<td><em>P. eryngii</em></td>
<td>VGGKLLGYVY 130</td>
</tr>
</tbody>
</table>

The name of each protein is as follows: *L. edodes*, ribosomal protein S8 (accession no. GAW05875.1) and *P. eryngii*, ribosomal protein S15a (accession no. KAF9498209.1).

Shaded characters: matched amino acid residues between two mushroom proteins.

Underlined characters: identified peptides by mass spectrometry and ProteinPilot™ analysis.