952 Purification of natural and recombinant melon profilin using affinity chromatography column

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Background: Profilin is one of actin binding proteins and has been identified as one of the common allergens in pollens and fruits. Different methods have been developed in purifying specific proteins, such as affinity chromatography.

Objectives: Affinity chromatography was chosen to purify profilin. Separation of profilin could be done by the high affinity of the allergen to IgG.

Methods: Recombinant profilin, generated by cDNA cloning, was expressed in *Escherichia coli* and purified with metal affinity chromatography. Purified rCuc m 2 was used to generate specific polyclonal antibody in rabbit. Rabbit IgG anti Cuc m 2 was affinity purified with protein A sepharose column. Purified IgG from previews step was used to develop (set up) an immunofinity column for purification of natural melon extract. rCuc m 2 was purified from melon extract, using glycine-HCl 0.2 M pH 2.8 as dilution buffer.

Result: Natural melon extract was purified to homogenous band from total melon extract by one step immunofinity chromatography. Recombinant melon profilin was purified with metal affinity chromatography. Purified proteins were characterized by SDS-PAGE and immunoblot.

Conclusion: The aim of this study was development of purification method for separation and characterization of natural and recombinant profilin for *in vivo* and *in vitro* diagnostic tests.
oral allergy syndrome after the ingestion of peach, melon, tomato, onion, strawberry, apple, banana, kiwi and nuts and contact eczema to natural rubber latex material. He always lived in the eastern region of Portugal, where high airborne pollen concentrations predominantly to grass, artemisia, parietaria and *Olea europea* can be found from March to July. Skin prick tests (SPT), serum specific IgE and immunoblotting assays were carried out in order to confirm the sensitisation and identify the molecular weight range of the proteins involved.

**Results:** He presented a high level of total IgE 2270 kU/L and the results of SPT and specific IgE are shown in the table. Immunoblotting assay results were as follows: Apple – IgE binding at 13.43 kDa band; Peanut – IgE binding at 29.59 kDa, three bands between 40.73 and 56.97 kDa; Peach: IgE binding at 82.49 kDa; Banana – IgE binding at 13.93 kDa; *Artemisia vulgaris* – IgE binding at 14.70 kDa; Latex – IgE binding at 12.41 kDa; The patient was medicated with inhaled budesonide 400 μg, nasal budesonide, cetirizine and terbutaline as needed. Avoidance of *Rosaceae* and *Cucurbitaceae* fruits, banana, kiwi, tomato and nuts was recommended. Specific immunotherapy to pollens (*Dactylis*, *Phleum* and *Parietaria*) was started in 2001 with clinical improvement. Immunoblotting inhibition assays between banana and latex revealed total inhibition of latex by banana extract and partial inhibition of banana by latex.

**Conclusions:** The results are compatible with a primary sensitisation to pollen grass, *Parietaria* and *Artemisia*, with clinical cross-reactivity with *Rosaceae* and *Cucurbitaceae*

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**Table 1** for abstract 954.

<table>
<thead>
<tr>
<th>Allergen</th>
<th>SPT (mm)</th>
<th>IgE (kU/L)</th>
<th>Allergen</th>
<th>SPT (mm)</th>
<th>IgE (kU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histamine</strong></td>
<td>5</td>
<td>–</td>
<td>Kiwi</td>
<td>5</td>
<td>10 (cl 3)</td>
</tr>
<tr>
<td><strong>Dactylis glomerata</strong></td>
<td>6</td>
<td>&gt; 100</td>
<td>Strawberry</td>
<td>2</td>
<td>17.7</td>
</tr>
<tr>
<td><strong>Poa pratensis</strong></td>
<td>16</td>
<td>&gt; 100</td>
<td>Peach</td>
<td>8</td>
<td>16.5</td>
</tr>
<tr>
<td><strong>Phleum pratensis</strong></td>
<td>8</td>
<td>68.2</td>
<td>Banana</td>
<td>6</td>
<td>17.5</td>
</tr>
<tr>
<td><strong>Parietaria officinalis</strong></td>
<td>7</td>
<td>68.2</td>
<td>Melon</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td><strong>Artemisia vulgaris</strong></td>
<td>8</td>
<td>4.7</td>
<td>Apple</td>
<td>6</td>
<td>–</td>
</tr>
<tr>
<td><strong>Triticum sativum</strong></td>
<td>14</td>
<td>–</td>
<td>Peanut</td>
<td>5</td>
<td>28 (cl 4)</td>
</tr>
<tr>
<td><strong>Corylus anellana</strong></td>
<td>6</td>
<td>–</td>
<td>Hazelnut</td>
<td>5</td>
<td>17.5</td>
</tr>
<tr>
<td><strong>Plantago major</strong></td>
<td>8</td>
<td>–</td>
<td>Carrot</td>
<td>4</td>
<td>25 (cl 4)</td>
</tr>
<tr>
<td><strong>Latex</strong></td>
<td>3</td>
<td>17.9</td>
<td>Celery</td>
<td>6</td>
<td>22.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(cl 4)</td>
<td>Onion</td>
<td>5</td>
<td>17.8</td>
</tr>
</tbody>
</table>

**Results:** Using polyclonal rabbit antiserum it was possible to detect all antigens from the common banana extract and also to compare the pattern of antigenicity between common banana and four banana cultivars analyzed in this study. Banana cultivars contain more or less the same protein profile, but there is a difference in the amount of proteins and isoforms thereof in the extracts. IgE antibodies from pooled patients sera were able to detect not only different isoforms of class I chitinase, but also β-1,3-glucanase. The difference in the amount of TLP was detected by patients’ IgE and rabbit polyclonal sera in the banana protein extracts.

**Discussion:** Rabbit polyclonal antiserum enabled us to analyze the presence and distribution of proteins and their isoforms in different banana cultivars. Results show that apart from the class I chitinase, which is known to be the major banana allergen, both β-1,3-glucanase and TLP can bind IgE antibodies from patients allergic to banana. This result suggests that fruit extracts could be replaced by well defined panel of relevant allergenic isoforms to achieve more reliable diagnostic reagents for food allergy.

**Background:** Banana fruit belongs to the *Musaceae* family and is extremely nutritious food, but also well known as an allergenic source, eliciting IgE-mediated reaction in sensitized individuals. Some banana proteins are proven to be allergens (profilin and class I chitinase) while the allergenic potential of other abundant proteins like thaumatin-like protein (TLP), β-1,3-glucanase and banana lectin has not yet been well studied. Evaluation of allergenic potential of individual proteins should indicate relevant allergens for the component-resolved allergy diagnosis.

**Methods:** Banana extracts from five different bananas were made using the standard procedure and analyzed for protein content by SDS-PAGE, IEF and 2D electrophoresis. After electrophoresis proteins were electrotransferred or printed to the nitrocellulose membrane. They were then analyzed with polyclonal antiserum obtained by immunization of the rabbit with common banana extract and also to common banana extract and 5% BSA conjugated antiserum. Patient samples from different European regions were analysed with this new reagent.

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**955 Banana allergens and protein distribution in different banana cultivars**

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**Background:** Allergy to fruit and vegetables is often associated with pollinosis. However, cross-reactivity patterns differ between geographical areas and climates depending on the differences in exposure to inhaled and ingested allergens. In central and northern Europe food allergy to fruits of the Rosaceae family is strongly associated with birch pollinosis because of the existence of allergens with Bet v 1 homology. In contrast, in the Mediterranean population allergic reactions to these fruits are more often related to lipid transfer proteins (LTPs). Bet v 1 analogues are labile and might be reduced by conventional extraction procedures of natural raw material. The ambition with this study was to explore new extraction buffer trying to find the optimal formula for conserving labile Bet v 1 analogues during extraction.

**Methods:** Six fruits, where the ImmunoCAP™ reagent showed less than optimal uptake for Bet v 1 sensitized patient samples, were selected. We produced extracts with a panel of extraction procedures, and coupled extracts from the optimal extract to an Improved ImmunoCAP™.

**Results:** Using polyclonal rabbit antiserum it was possible to detect all antigens from the common banana extract and also to compare the pattern of antigenicity between common banana and four banana cultivars analyzed in this study. Banana cultivars contain more or less the same protein profile, but there is a difference in the amount of proteins and isoforms thereof in the extracts. IgE antibodies from pooled patients sera were able to detect not only different isoforms of class I chitinase, but also β-1,3-glucanase. The difference in the amount of TLP was detected by patients’ IgE and rabbit polyclonal sera in the banana protein extracts.

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**956 Methods for improving natural fruit extracts**

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1 MIAB, Research & Development, Uppsala, Sweden, 2 Phadia AB, Medical Department, Uppsala, Sweden

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