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# Characterization of major allergens of royal jelly *Apis mellifera*

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**Abstract.** Royal jelly is widely consumed in the community and has perceived benefits ranging from promoting growth in children and improvement of general health status to enhancement of longevity for the elderly. However, royal jelly consumption has been linked to contact dermatitis, acute asthma, anaphylaxis and death. High prevalence of positive skin tests to royal jelly have been reported among atopic populations in countries with a high rate of royal jelly consumption. The present study is aimed to identify the major allergens of royal jelly. Royal jelly extract was separated by sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) and 2-dimensional electrophoresis (2-D). Immunoblotting of the SDS-PAGE and 2-D profiles were performed to identify the allergenic spots. Spots were then excised from the 2-D gel, digested with trypsin and analyzed by mass spectrometry. The SDS-PAGE of royal jelly extract revealed 18 bands between 10 to 167 kD. Western blot of the fractionated proteins detected 15 IgE-binding bands between 14 to 127 kD with seven major allergens of 32, 40, 42, 49, 55, 60 and 67 kD using serum from 53 subjects with royal jelly allergy. The 2-D gel fractionated the royal jelly proteins to more than 50 different protein spots. Out of these, 30 spots demonstrated specific IgE affinity to the sera tested. Eight spots of the major royal jelly allergens were selected for mass-spectrometry analysis. Digested tryptic peptides of the spots were compared to the amino acid sequence search in protein databases which identified the fragments of royal jelly homologus to major royal jelly protein 1 (MRJ1) and major royal jelly protein 2 (MRJ2). In conclusion, the major allergens of royal jelly are MRJ1 and MRJ2 in our patients' population.

## INTRODUCTION

Royal jelly is a yellowish-white, creamy, acidic secretion, with a slightly pungent odor and taste produced by the hypopharyngeal and mandibular glands of worker honey bees (*Apis mellifera*) (Takahama & Shimazu, 2006). Royal jelly is widely consumed in the community and has perceived benefits ranging from promoting growth in children and improvement of general health status to enhancement of longevity for the elderly (Leung *et al.*, 1997a). Royal jelly has a much larger market in Asia than in the US or Europe, and in Asia it is commonly found in products including cosmetics, food supplements, beverages and is used in commercial medical products (FAO, 2007). The world's largest producer and exporter of royal jelly is China which accounts for approximately 60% of world production, followed by Korea, Taiwan and Japan (FAO, 2007).

However, royal jelly consumption has been linked with contact dermatitis (Takahashi *et al.*, 1983), acute asthma, anaphylaxis and death (Peacock *et al.*, 1995; Thien *et al.*, 1996; Leung *et al.*, 1997a; Lombardi *et al.*, 1998; Takahama & Shimazu, 2006). High prevalence of positive skin tests to royal jelly has also been reported among atopic populations in countries with a high

rate of royal jelly consumption (Leung *et al.*, 1997a). In Hong Kong, the prevalence of skin prick test (SPT) reactivity among adult asthmatic patients was 16.8% (Leung *et al.*, 1997b) and 7.4% among hospital employees (Leung *et al.*, 1997a).

Several studies have shown that royal jelly-sensitive subjects possess serum IgE antibodies to a number of royal jelly components. Royal jelly proteins with molecular weight ranging from 25 to 55 kD have been detected as IgE-binding proteins and components of 47 kD and 55 kD have been recognized as the major allergens of royal jelly (Thien *et al.*, 1996; Leung *et al.*, 1997b). Other minor allergens were also detected at ~39, 40, 60, 67 and 94 kD (Thien *et al.*, 1996; Leung *et al.*, 1997b).

In Malaysia, royal jelly is marketed widely and used by all age groups. An earlier study among atopic patients demonstrated that royal jelly sensitivity is present in this country (unpublished data). Thus, the present study is aimed to identify the major allergens of royal jelly using hypersensitive patients' sera to royal jelly by proteomics methods.

## MATERIAL AND METHODS

### Allergen Extraction

Royal jelly extract was prepared according to the procedures described by Thien *et al.* (1996) with minor modification. Briefly, 5g royal jelly was mixed in 10 ml distilled water and rotated overnight at 4°C before centrifuging. After centrifugation, the supernatant was filtered and dialyzed against distilled water for 48 hours. The extract was then lyophilized and stored at -20°C. Protein content was determined with the Total Protein Kit (Sigma, Germany).

### Allergen Testing and Collection of Sera

Sera from 53 patients with history of allergy and positive skin prick test (SPT) to royal jelly extract were used in this study. The SPT was performed by a medical officer at the Ear, Nose and Throat (ENT) Clinic of Hospital Kuala Lumpur (HKL). This project was approved by Ethics Committee of Ministry of Health of Malaysia (MOH).

### SDS-PAGE (Sodium Dodecyl Polyacrylamide Gel Electrophoresis)

Protein profile of royal jelly extract was determined by SDS-PAGE using the method described by Thien *et al.* (1996) with minor modification. The sample was resolved in a 12% separating gel with a 4% stacking gel by using a Mini Protean 3 Apparatus (BioRad, USA). The individual protein bands were then identified with Coomassie blue. Molecular weight standards (Fermentas, Germany) were used to estimate the molecular weights of the royal jelly proteins using an imaging densitometer (BioRad, USA).

### IgE-binding Proteins

Immunoblotting was performed to identify the IgE-binding components of royal jelly using sera from 53 patients with positive skin prick test (SPT) to royal jelly extract. In brief, the separated proteins of royal jelly were electrophoretically transferred from unstained SDS-PAGE gel to 0.45 mm pore size nitrocellulose membrane using a Mini Transblot System (BioRad, USA). The nitrocellulose blot was then cut, washed with tris-buffered saline (TBS) containing Tween 20 (TTBS) and then blocked with 5% non fat milk in TBS. The blocked strips were then incubated overnight with individual patient's serum. The bound IgE on the strips was detected by incubation with biotinylated goat antihuman IgE (Kirkergaard & Perry Laboratories, UK) followed by incubation in streptavidin-conjugated alkaline phosphatase (BioRad, USA). Finally, the Alkaline Phosphatase Conjugate Substrate Kit (BioRad, USA) was used to detect the bound IgE.

### 2-Dimensional Electrophoresis (2-DE) and immunoblotting

For 2-D gel electrophoresis, the lyophilized royal jelly extract was resuspended in rehydration buffer. 50 µg of protein extract was applied to 7 cm of immobilized pH 3-10 non-linear gradient strip (BioRad, USA) for rehydration overnight and focusing using IEF cell (BioRad, USA). The first dimensional

electrophoresis was performed to separate the proteins by charge with 4 steps: 100 V for 1 minute, 250 V for 30 minutes, 4000 V for 2 hours and 4000 V for 10000 V-hr (V-hour). The strips were fractionated by molecular weight using 12% of separating gel of SDS-PAGE. Protein spots profile was visualized with the use of Coomassie blue. The major IgE-reactive protein spots of royal jelly extract were identified by immunoblotting using sera of 10 subjects known to have specific IgE to royal jelly in 1-D immunoblotting.

**Tryptic digestion of 2-DE Spots, MALDI-ToF MS, Q-ToF MS and Database Search**

The Coomassie-stained protein spots corresponding to those recognized by the above sera were manually excised and subjected to destaining, reduction, carbamidomethylation prior to in-gel tryptic digestion on a MassPrep workstation (Micromass, UK) using modified porcine trypsin (Promega, USA).

Peptide Mass Fingerprints were obtained using a 5% aliquot of the samples, analysed on a MALDI-ToF instrument (M@LDI from Micromass, UK). The remaining extracts were concentrated in a Speed Vac and reconstituted in 1% aqueous formic acid. This was subjected to LC-MS/MS analysis, performed on a CapLC/QToF II instrument (Micromass, UK). All fragment ion (MS/MS) spectra were searched against the MSDB database using the Mascot search tool (Matrix Science).

## RESULTS

**Royal Jelly Proteins**

Figure 1 (A) shows a SDS-PAGE profile of royal jelly proteins stained with coomassie blue. SDS-PAGE of royal jelly proteins revealed approximately 18 bands in the range between 10 to 167 kD, with a heavy cluster in the molecular weight region of 25-72 kD. Proteins at 40 to 55 kD were heavily stained in the separation profile.

**IgE-binding Proteins**

Immunoblotting studies detected 50/53 (94.3%) of sera from patients with royal jelly allergy show IgE reactivity with the proteins

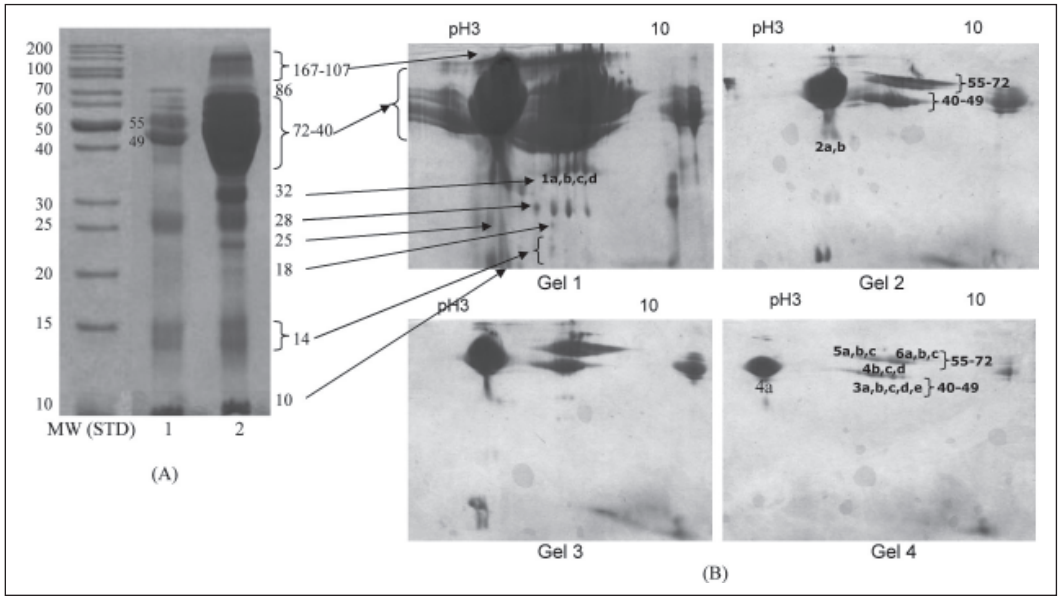


Figure 1: (A) Protein profile of royal jelly proteins separated by means of SDS-PAGE; and (B) 2-dimensional electrophoresis and stained with coomassie blue. Lane 1 and 2 are SDS-PAGE profiles of10 mg/ml and 50 mg/ml of royal jelly extract, respectively, while gel 1, 2, 3 and 4 are 2-D protein profiles of 50 mg/ml, 20 mg/ml, 10 mg/ml and 1 mg/ml of royal jelly extract, respectively. MW is molecular weight protein marker in kiloDalton (kD).

in the range of 14 to 127 kD (Figure 2 and Table 1). All subjects (100%) demonstrated IgE affinity to the 40, 42, 49 and 55 kD and thus identified as major allergens. Major allergens were also detected at 32 kD (74%), 60 kD (78%) and 67 kD (86%). IgE-binding proteins were also detected at 14, 15, 25, 28, 30, 86, 107 and 127 kD, but only as minor allergens. In total, the sera from the subjects revealed 15 IgE antibody-reactive bands in royal jelly extract. No IgE-binding was observed when sera from non-allergic subjects were used in the immunoblotting.

**2-DE Profiles**

Figure 1 (B) shows 2-dimensional maps of the royal jelly proteins at four different concentrations. About 50 distinct spots with molecular masses from 10 to 167 kD and pI between 3 to 10 were visible with coomassie blue staining.

**IgE-binding Spots**

The 2-D gels were blotted and incubated with the sera of 10 patients identified to have specific IgE to royal jelly. The IgE-stained patterns of the royal jelly protein spots obtained with all the sera demonstrated almost similar patterns and 4 of these immunoblots are shown in Figure 3. Immunoblot results are summarized in Table 2. Most IgE-binding spots of royal jelly major allergens had a pI above pH 4.0 and a molecular mass between 32 to 67 kD. Immunoblots revealed up to 30 different immunostained spots. The most abundant IgE-binding spots of royal jelly major allergens were spot numbers 2a to 4d. One spot from major allergens of 32, 40, 60 and 67 kD; and two spots from major allergens of 42-49 and 55 kD were selected for mass spectrometry analysis. No IgE-binding spots were detected by immunoblotting using a control sera from a non-allergic subject.

**Allergen Identification**

Eight Coomassie-stained protein spots (1d, 2a, 3c, 3e, 4a, 4b, 5b and 6c) were analyzed after tryptic digestion by mass spectrometry. The peptide mass fingerprints matched significantly with known royal jelly proteins. The results of the spot identification are reported in Table 3. Spots 1d, 2a, 3c and 3e have been identified as major royal jelly protein 2 (recently known as apalbumin 2), while spots 4a, 4b, 5b and 6c are all major royal jelly protein 1 (apalbumin 1). All the identified proteins belonged to *Apis mellifera*.

## DISCUSSION

IgE-mediated allergy to royal jelly has been documented (Thien *et al.*, 1996; Leung *et al.*, 1997b; Takahama & Shimazu, 2006). However, studies on the identification of royal jelly allergens are very limited. The

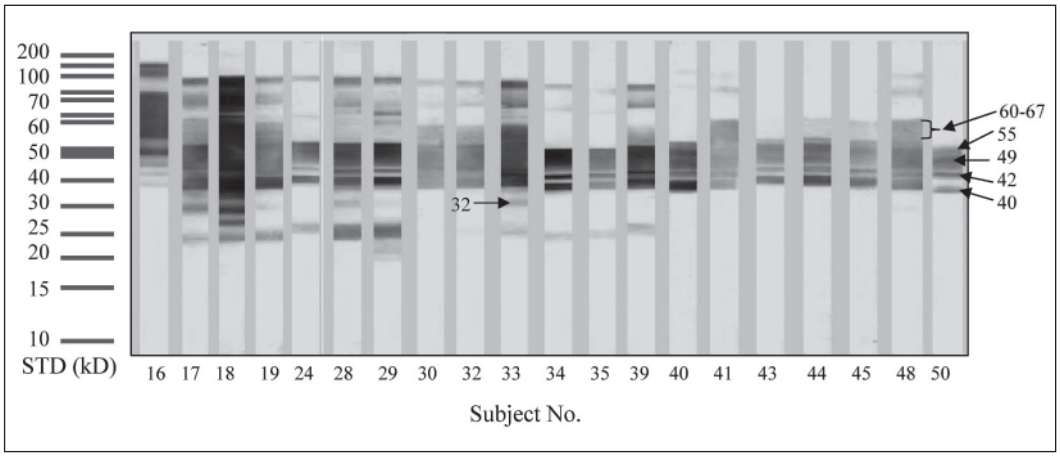


Figure 2: Immunoblotting results of 20 royal jelly-sensitized patients. STD is a molecular weight marker in kiloDalton (kD).

Table 1: IgE-binding proteins of 50 royal jelly-sensitized patients (subjects no. 1-50)

| | Royal jelly allergen fraction and estimated molecular weight (kD) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Subject no.** | **1 127** | **2 107** | **3 86** | **4 67** | **5 60** | **6 55** | **7 49** | **8 42** | **9 40** | **10 32** | **11 30** | **12 28** | **13 25** | **14 18** | **15 14** |
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| 2 | | ▲ | ▲ | ▲ | ▲ | ▲ | ▲ | ▲ | ▲ | ▲ | | | | | |
| 3 | | ▲ | ▲ | ▲ | ▲ | ▲ | ▲ | ▲ | ▲ | ▲ | | | | | |
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| 50 | | | | | | ▲ | ▲ | ▲ | ▲ | | | | | | |
| **n** | **1** | **24** | **24** | **43** | **39** | **50** | **50** | **50** | **50** | **37** | **8** | **13** | **7** | **15** | **6** |
| **%** | **2** | **48** | **48** | **86*** | **78*** | **100*** | **100*** | **100*** | **100*** | **74*** | **16** | **26** | **14** | **30** | **12** |

▲ IgE binding proteins

* Major allergens

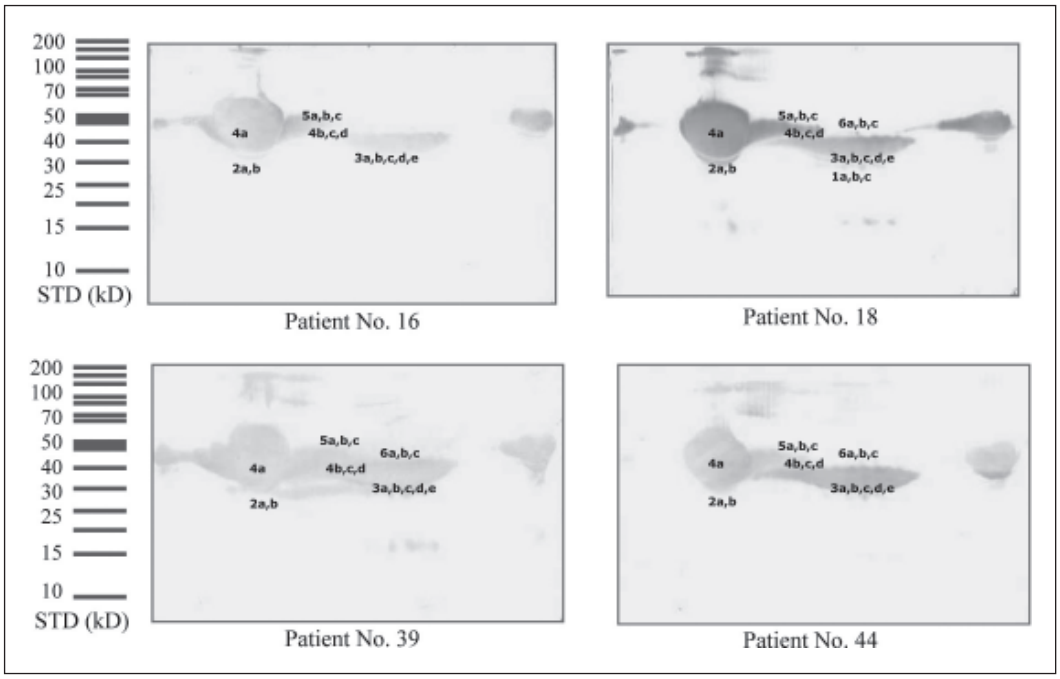


Figure 3: Immunoblots profile of 2-D gels using serum of 4 patients. STD are molecular weight markers (kD).

Table 2: Summary of IgE binding spots of the major royal jelly allergens in 2-dimensional immunoblots

| Spot No. | MW (kD) | pI | Subject No. | | | | | | | | | | Positive (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 16 | 17 | 18 | 28 | 32 | 33 | 34 | 39 | 44 | 48 | |
| **1a** | 32 | 4.9 | | + | + | + | | + | | | | + | 50 |
| **1b** | 32 | 5.0 | | + | + | + | | + | | | | + | 50 |
| **1c** | 32 | 5.1 | | + | + | + | | + | | | | + | 50 |
| ***1d** | 32 | 5.2 | + | + | + | + | + | + | + | + | + | + | 100 |
| ***2a** | 40 | 4.0 | + | + | + | + | + | + | + | + | + | + | 100 |
| **2b** | 40 | 4.2 | + | + | + | + | + | + | + | + | + | + | 100 |
| **3a** | 42-49 | 4.9 | + | + | + | + | + | + | + | + | + | + | 100 |
| **3b** | 42-49 | 5.0 | + | + | + | + | + | + | + | + | + | + | 100 |
| ***3c** | 42-49 | 5.1 | + | + | + | + | + | + | + | + | + | + | 100 |
| **3d** | 42-49 | 5.2 | + | + | + | + | + | + | + | + | + | + | 100 |
| ***3e** | 42-49 | 5.3 | + | + | + | + | + | + | + | + | + | + | 100 |
| ***4a** | 55 | 4.0 | + | + | + | + | + | + | + | + | + | + | 100 |
| ***4b** | 55 | 4.7 | + | + | + | + | + | + | + | + | + | + | 100 |
| **4c** | 55 | 4.8 | + | + | + | + | + | + | + | + | + | + | 100 |
| **4d** | 55 | 4.9 | + | + | + | + | + | + | + | + | + | + | 100 |
| **5a** | 60 | 4.7 | + | + | + | + | + | + | | + | + | + | 90 |
| ***5b** | 60 | 4.8 | + | + | + | + | + | + | | + | + | + | 90 |
| **5c** | 60 | 5.0 | + | + | + | + | + | + | | + | + | + | 90 |
| **6a** | 67 | 5.2 | + | + | + | + | + | + | | + | + | + | 90 |
| **6b** | 67 | 5.3 | + | + | + | + | + | + | | + | + | + | 90 |
| ***6c** | 67 | 5.4 | + | + | + | + | + | + | | + | + | + | 90 |

\+ IgE-binding spot
* Spot selected for mass spectrometry analysis

Table 3: Profound search for mass fingerprint results of royal jelly major allergens

| Spot No. | Molecular mass and pI of matched proteins: Theoretical/ found | No. match peptides/total signals | Coverage of protein sequence | Protein description |
|---|---|---|---|---|
| **1d** | 51.441/ 326.83/ 5.2 | 14/108 | 38% | Major Royal jelly Protein 2 (Apalbumin-2) (Bee-milk protein), *Apis mellifera* **AAC61894** |
| **2a** | 51.441/ 406.83/ 4.0 | 15/136 | 35% | Major Royal jelly Protein 2 (Apalbumin-2) (Bee-milk protein), *Apis mellifera* **AAC61894** |
| **3c** | 51.441/ 42-496.83/ 5.1 | 16/162 | 40% | Major Royal jelly Protein 2 (Apalbumin-2) (Bee-milk protein), *Apis mellifera* **AAC61894** |
| **3e** | 51.441/ 42-496.83/ 5.3 | 14/144 | 33% | Major Royal jelly Protein 2 (Apalbumin-2) (Bee-milk protein), *Apis mellifera* **AAC61894** |
| **4a** | 49.311/ 555.10/ 4.0 | 21/207 | 56% | Major Royal jelly Protein 1 (Apalbumin-1) (Bee-milk protein), *Apis mellifera* **AAC61895** |
| **4b** | 49.311/ 555.10/ 4.7 | 15/136 | 35% | Major Royal jelly Protein 1 (Apalbumin-1) (Bee-milk protein), *Apis mellifera* **AAC61895** |
| **5b** | 49.311/ 605.10/ 4.8 | 21/211 | 60% | Major Royal jelly Protein 1 (Apalbumin-1) (Bee-milk protein), *Apis mellifera* **AAC61895** |
| **6c** | 49.311/ 675.10/ 5.4 | 18/158 | 45% | Major Royal jelly Protein 1 (Apalbumin-1) (Bee-milk protein), *Apis mellifera* **AAC61895** |

present study was performed to identify the IgE-binding proteins of royal jelly. SDS-PAGE profile demonstrated 18 protein fractions with molecular weight in the range of 10 to 167 kD, and a heavy cluster between 25 to 72 kD. A similar protein pattern was found by other studies (Thien *et al.*, 1996; Leung *et al.*, 1997b).

Our study demonstrated that subjects with clinical sensitization to royal jelly have serum IgE antibodies to a number of royal jelly components. 15 IgE-binding proteins were recognized in this study. Four proteins of molecular weight 40, 42, 49 and 55 kD were recognized by sera of all subjects, suggesting that these four components are the major allergenic proteins of royal jelly. Interestingly, the 55 kD protein was also detected by all sera from royal jelly-sensitive subjects examined by Thien *et al.* (1996). In addition, our study also detected another three major allergens of 32, 60 and 67 kD. All these proteins have been detected as minor allergens by other studies (Thien *et al.*, 1996; Leung *et al.*, 1997b).

Our 2-D protein profile revealed about 50 royal jelly protein spots within the acidic and alkaline range. However all the IgE-binding spots were located in the acidic range between pI of 4.0 to 5.4. This 2-D pattern is similar to the study reported by Scarselli *et al.* (2005). In the present study, the tryptic peptide fragments isolated from the digested 55, 60 and 67 kD spots are similar to the segments of major royal jelly protein 1 (MRJP 1), while the spots at 32, 40, 42 and 49 kD are similar to major royal jelly protein 2 (MRJP 2) segments. Thien *et al.* (1996) and Leung *et al.* (1997b) have suggested that major royal jelly allergens

may be 'major royal jelly proteins (MRJPs)' and our findings have confirmed this. To our knowledge, this is the first time MRJPs were identified as royal jelly allergens by the proteomics approach.

To date, nine members of MRJPs (49-87 kD) family, now named apalbumins (Scarselli *et al*., 2005) have been identified, and five of them (MRJP 1 to MRJP5) represent about 82 to 90% of the total protein content of royal jelly (Schmitzova *et al*., 1998; Simuth, 2001). MRJP1 is the most abundant and best characterized glycoprotein of royal jelly (Albert & Klaudiny, 2004). MRJP 1 represents 48% of the water soluble proteins of royal jelly, and appears as a single protein on SDS-PAGE with molecular weight of 55 kD (Schmitzova *et al*., 1998; Simuth, 2001). With isoelectrofocusing, it shows at least eight isoelectrophoretic variants (Simuth, 2001). MRJP1 not only functions as a component of larval food but it also plays a role in the honeybee's brain (Simuth, 2001).

MRJ2 is the third most abundant royal jelly protein after MRJP1 and MRJP3. Based on SDS-PAGE analysis, MRJP2 has a molecular weight of 49 kD (Srisuparbh *et al*., 2003). It should be noted that spot 1c and 2b with molecular mass of 32 and 40 kD, respectively were identified as MRJP2 in this study, suggesting that the two proteins are degradation products of MRJP2 due to a proteinase activity, as reported by Scarselli *et al*. (2005).

In conclusion, our study demonstrated that the major IgE-binding proteins for royal jelly were proteins between 32 to 67 kD corresponding to MRJP 1 and MRJP 2. This finding shows that MRJPs are important in allergy to royal jelly among our local patients.

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REFERENCES

Albert, S. & Klaudiny, J. (2004). The MRJP/YELLOW protein family of *Apis mellifera*: Identification of new members in the EST library. *Journal of Insect Physiology* **50**: 51-59.

Food and Agriculture Organization of the United Nations (FAO). (2007). Value-added products from beekeeping. URL: www.fao.org/docrep/w0076E/w0076E00.htm.

Leung, R., Ho, A., Chan, J. & Lai, C.K.W. (1997a). Royal jelly consumption and hypersensitivity in the community. *Clinical Experimental Allergy* **27**: 333-336.

Leung, R., Thien, F.C.K., Baldo, B. & Czarny, D. (1997b). Royal jelly-induced asthma and anaphylaxis: Clinical characteristics and immunological correlations. *Journal Allergy Asthma & Immunology* **96**:1004-1007.

Lombardi, C., Senna, G.E., Gatti, B., Feligioni, M., Riva, G., Bonadonna, P., Damma, A.R., Canonica, G.W. & Passalacqua, G. (1998). Allergic reactions to honey and royal jelly and their relationship with sensitization to compositae. *Allergologia et immunopathologia* **26**(6): 288-290.

Peacock, S., Murray, V. & Turton, C. (1995). Respiratory distress and royal jelly. *British Medical Journal* **311**(7018): 1472.

Scarselli, R., Donadio, E., Giuffrida, M.G., Fortunato, D., Conti, A., Balestreri, E., Felicioli, R., Pinzauti, M., Sabatini, A.G. & Felicioli, A. (2005). Toward royal jelly proteome. *Proteomics* **5**: 769-776.

Schmitzova, J., Klaudiny, J., Albert, S., Schroder, W., Schreckengost, W., Hanes, J., Judova, J. & Simuth, J. (1998). A family of major royal jelly proteins of the honeybee *Apis mellifera* L. *Cellular and Molecular Life Sciences* **54**: 1020-1030.

Simuth, J. (2001). Some properties of the main protein of honeybee (*Apis mellifera)* royal jelly. *Apidologie* **32**: 69-80.

Srisuparbh, D., Klinbunga, S., Wongsiri, S. & Sittipraneed, S. (2003). Isolation and characterization of major royal jelly cDNAs and proteins of honey bee (*Apis cerana*). *Journal of Biochemistry and Molecular Biology* **36**(6): 572-579.

Takahashi, M., Matsuo, I. & Ohkido, M. (1983). Contact dermatitis due to honeybee royal jelly. *Contact Dermatitis* **9**: 452-455.

Takahama, H. & Shimazu, T. (2006). Food-induced anaphylaxis caused by ingestion of royal jelly. *Journal Dermatology* **33**: 424-426.

Thien, F.C.K., Leung, R., Baldo, B.A., Weiner, J.A., Plomley, R. & Czarny, D. (1996). Asthma and anaphylaxis induced by royal jelly. *Clinical Experimental Allergy* **26**: 216-222.