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Cloning and expression of Aca f 1: a new allergen of *Acacia farnesiana* pollen

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Abstract

Acacia farnesiana is the main source of allergenic pollen and one of the most important causes of respiratory allergic disease in tropical and subtropical regions of the world. The purpose of this study was to produce a recombinant variety of allergenic Ole e 1-like protein from the pollen of this tree. To predict its allergenic cross-reactivity with other members of the Ole e 1-like protein family of common allergenic plants, the nucleotide sequence homology of the Acacia Ole e 1-like protein was evaluated. Amplification of cDNA strands encoding Acacia Ole e 1-like protein was performed by polymerase chain reaction (PCR) and sequenced. Following expression in Escherichia coli using the pET-21b(+) vector, the recombinant protein was purified using metal-affinity chromatography. IgE-binding competence of purified recombinant Ole e 1- like protein (rAca f 1) was analysed by immunoassay using 25 sera collected from Acacia pollen-sensitised patients. Nucleotide sequencing revealed an open reading frame of 453 bp encoding 150 amino acid residues that belonged to the Ole e 1-like protein family, and 11 patients (44%) had considerable specific IgE levels for the rAca f 1. Immunodetection and inhibition assays indicated that the purified rAca f 1 may be the same as that in the crude extract. Aca f 1, the second allergen from Acacia pollen, was identified as a member of the family of Ole e 1-like protein. A high degree of homology was found among amino acid sequences of Aca f 1 and several allergenic members of Ole e 1-like protein family.

Key words: *cloning, expression, Acacia, Aca f 1.*

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Introduction

Acacia farnesiana (*Vachellia farnesiana*), a member of the *Fabaceae* family, is a widespread tree throughout the tropical and subtropical regions of the United States, Asia, Africa, and Australia [1, 2]. Pollens from *Acacia* have been reported as a main source of pollinosis in subtropical countries such as Iran, Saudi Arabia, and the United Arab Emirates, where the frequency of sensitisation ranges from 25% to 48% [3-8].

Immunochemical analysis of the protein profile of *Acacia* pollen indicated several components ranging from 12 to 105 kDa. Moreover, the 15, 45, 50, 66, and 85 kDa proteins are recognised as dominant IgE-binding components of *Acacia* pollen [1, 9, 10]. A significant IgE cross-reactivity between the *Acacia* tree and other plants, particularly *Prosopis juliflora* (mesquite) and *Lolium perenne* (rye grass) pollen allergens, has also been described [1, 11, 12]. In a recent study, Aca f 2, the first allergen from *A. farnesiana* pollen, was identified as belonging to the family of profilins [8].

Despite a high prevalence of sensitisation to *Acacia* pollen in semiarid countries, there is little information about the molecular characterisation of *A. farnesiana* pollen allergens. Here we report Aca f 1, a new allergen from *Acacia* pollen, which is a member of the Ole e 1-like protein family. The first allergenic member of this family was isolated from *Olea europaea* pollen as the major allergen of olive pollen (Ole e 1) [13]. In previous studies, similar allergens were also identified in other plants such as *Fraxinus excelsior* (Fra e 1) [14], *Ligustrum vulgare* (Lig v 1) [15] *Syringa vulgaris* (Syr v 1) [16], *Salsola kali* (Sal k 5) [17], and *Chenopodium album* (Che a 1) [18].

In this study, we expressed and purified Aca f 1 in *Escherichia coli*, and subsequently evaluated the immunoreactivity of this recombinant allergen. Moreover, the nucleotide sequence homology of Aca f 1 was investigated with the most common allergenic Ole e 1-like proteins in tropical regions.

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Material and methods

Pollen collection and crude extract preparation

Pollen was collected from the plants during pollination season (February-May) from different areas in and around Ahvaz city, a tropical region in southwest Iran. Collection and processing of pollen materials were performed with care by trained pollen collectors according to previous studies [19, 20]. Pollen materials were defatted using repeated changes of diethyl ether. Pollen was extracted as defined previously [21]. In brief, two grams of pollen were mixed with 10 ml of phosphate-buffered saline (PBS) 0.01 M (pH 7.4) by continuous stirring for 18 hours at 4°C. The supernatant was separated by centrifugation at 14,000 × *g* for 30 minutes, filtered, and the supernatant collected. The extract was then freeze-dried. The protein content of the extract was measured using the method of Bradford [22]. Finally, the extract was freeze-dried and stored at –20°C for later use in the current study.

Study population and skin prick tests (SPTs)

Twenty-five patients with SPT positive for *Acacia* pollen and respiratory allergy history participated in this study. Five additional subjects who showed negative SPT responses and no specific IgE to *A. farnesiana* pollen extract were included as negative controls. All patients and controls provided informed consent. SPTs were performed by an experienced nurse under a physician's supervision, and patients with a positive SPT donated a serum sample. Afterwards, patients' serum samples were immediately stored at –20°C before use.

Evaluation of total and specific IgE

Total serum IgE levels were measured using a commercially available ELISA kit according to the manufacturer's instructions (Euroimmun, Lübeck, Germany). For the detection of specific IgE against *A. farnesiana* pollen proteins in allergic patients, an indirect ELISA was developed. For this purpose, 3 µg/well of *A. farnesiana* pollen extract in 100 µl carbonate buffer (15 mM Na_2CO_3 and 35 mM $NaHCO_3$, pH 9.6) was incubated at 4°C overnight in a 96-well ELISA microplate (Nunc A/S, Roskilde, Denmark). The plates were blocked with 150 µl of PBS-2% bovine serum albumin (BSA) solution (1 hour at 37°C) and then incubated with 100 µl of patients' sera for three hours at room temperature with shaking. Each well was then incubated for two hours at room temperature with a 1 : 500 dilution of biotinylated goat anti-human IgE antibody (Nordic-MUbio, Susteren, Netherlands) in 1% BSA. Following five gentle washes with T-PBS (PBS containing 0.05% Tween 20) 100 µl of a 1 : 10,000 dilution of horseradish peroxidase-conjugated streptavidin (Bio-Rad laboratories, Hercules, CA, USA) (diluted in PBS – 1% BSA) and incubated for one hour at room temperature. After an additional washing step, 100 µl of a tetramethylbenzidine (TMB-H_2O_2; Sigma-Aldrich, St. Louis, MO) substrate solution was used as the HRP-substrate. Following a 20-minute incubation in the dark, the reaction was stopped by the addition of 100 µl of 3 M HCl. Then, the optical density (OD) in each well was determined at 450 nm using an ELISA reader. OD three times greater than the mean values of three determinations of pooled sera from negative controls (i.e. > 0.16 OD units) was considered to be positive.

Amplification of Aca f 1 gene and sequencing

Following the isolation of total RNA and synthesis of cDNA, the coding sequence of Aca f 1 was cloned using a degenerate forward primer and reverse primer used for cDNA amplification, designed according to consensus nucleotide sequences for reported allergens from the Ole e 1-like protein family, which had a high degree of amino acid sequence identity [14, 17, 18, 23-25]. These include the forward and reversed primers 5′-ACKATKTTYCCMAACCTCCA-3′ and 5′-TTAATTAGCTTTAACATCATAAAGATCC-3′, respectively.

The amplified fragment was ligated into a pTZ57R/T TA cloning vector using the InsTAclone™ PCR Cloning Kit (Thermo Scientific, Waltham, MA, USA), according to the manufacturer's instructions. *Escherichia coli* TOP10 cells (Invitrogen, Carlsbad, CA, USA) were transformed with the recombinant pTZ57R/T vector using the manufacturer's protocol. Recombinant plasmid was subsequently purified from the gel using a Plasmid Extraction Kit (GeNet Bio, Chungnam, Korea) and sequenced using the dideoxy chain termination method at Bioneer Inc. (Daejeon, Korea). The DNA sequence of Aca f 1 was submitted to the NCBI GenBank database of (http://www.ncbi.nlm.nih.gov/) under accession number KR870435.

Production and purification of recombinant Aca f 1 (rAca f 1) allergen

Specific primers for the amplification of Aca f 1 cDNA were designed using the obtained sequence of Aca f 1. These contained overhangs with *Not* I and *Xho* I restriction sites for direct cloning into expression plasmid pET-21b(+) (Novagen, Gibbstown, NJ, USA) as follows: the sense primer (5′-TCC**GCGGCCGC**ACKATKTTYCCMAACCTCCA-3′, *Not* I restriction site is bolded) and the antisense primer (5′-CC**CTCGAG**TTAATTAGCTTTAACATCATAAAGAT-3′, *Xho* I restriction site is bolded). After PCR amplification, the resulting product was digested with *Not I* and *Xho* I restriction enzymes according to the manufacturer's protocol (Thermo Scientific, Waltham, MA, USA). After digestion of the PCR product and ligation into the digested pET-21b (+) plasmid with the same enzymes, correct constructs were transformed into competent *E. coli* BL21 (DE3) cells (Novagen, Gibbstown, NJ, USA). Subsequently,

a clone of recombinant plasmid pET-21b(+)/Aca f 1 was inoculated into 2 ml of Lysogeny broth (LB) medium containing 100 µg/ml of ampicillin and incubated at 37°C. Expression of rAca f 1 was induced by isopropyl β-D thiogalactopyranoside (IPTG) to a concentration of 0.5 mM. Subsequently, the cells were isolated by centrifugation (3500 × *g*, 20 minutes, 4°C), resuspended in lysis buffer (50 mM Tris–HCl pH 6.8, 15 mM imidazole, 100 mM NaCl, 10% glycerol, and 0.5% Triton X-100) and then disrupted by sonication. Purification of rAca f 1 was performed using Ni-NTA affinity chromatography (Invitrogen, Carlsbad, CA, USA) from the soluble phase of the lysate, following the manufacturer's instructions.

Quantification of Specific IgE to rAca f 1 by ELISA

An indirect ELISA was developed for the determination of levels of specific IgE against rAca f 1 using *Acacia* allergic patients' sera, as previously reported [26]. The wells of the ELISA microplate were coated with 100 µl/well of purified recombinant rAca f 1 (3 µg/ml).

IgE-binding reactivity of rAca f 1 by immunoblotting assay

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of *Acacia* pollen extract was performed using 12.5% acrylamide separation gels as described previously [8]. The protein bands from the electrophoresis of *Acacia* pollen extract or purified rAca f 1 were electro-transferred to polyvinylidene difluoride (PVDF) membranes (GE Healthcare, UK), and after blocking and washing, membranes were incubated with a serum pool or individual sera from patients with *Acacia* allergy or with control sera (1 : 5 dilutions) for three hours. Biotinylated goat anti-human IgE (Nordic-Mubio, Susteren, Netherlands) (1 : 1000 v/v in PBS) was added to detect specific IgE, and the blotted membrane strips were then incubated with 1 : 12,000 v/v in TPBS-HRP-linked streptavidin (Sigma-Aldrich, St. Louis, Mo, USA). Finally, strips were incubated with Super signal West Pico Chemiluminescent Substrate Kit (Thermo Scientific, Waltham, MA, USA) for five minutes, and proteins were then visualised by chemiluminescence using ChemiDoc XRS+ system (Bio-Rad).The molecular masses of protein bands were estimated using Image Lab Analysis Software (Bio-Rad) by comparison with protein markers of known molecular weights (Amersham Low molecular weight Calibration Kit for SDS electrophoresis, GE Healthcare, UK).

Evaluation of cross-reactivity using *in vitro* inhibition assays

ELISA inhibition assay were performed as reported previously [19, 20]. In this method a pooled serum (1 : 2 v/v) from hyper-reactive *Acacia* allergic patients (No. 1, 3, 8, and 9) was pre-incubated for overnight at 4°C with either 1000, 100, 10, 1, 0.1, or 0.01 µg of rAca f 1 as inhibitors or with BSA as a negative control before use. The percentage inhibition was calculated using the following formula: (OD of sample without inhibitor-OD of sample with inhibitor/OD of sample without inhibitor) × 100.

To study cross-inhibition between natural and recombinant Aca f 1, a mixture of 100 µl of pooled serum (1 : 5 v/v) was incubated with natural *Acacia* pollen extract (15 µg/ml, as inhibitor), rAca f 1 (8 µg/ml, as inhibitor), or BSA (as negative control) overnight at 4°C with shaking. Preincubated sera were used to assess the reactivity of a PVDF membrane blotted with natural *Acacia* pollen extract and rAca f 1.

Table 1. Clinical characteristics of the patients, skin prick test reactivity, and specific IgE values to recombinant Aca f 1

Aca f 1-reactive patients	Age (years)/sex	Clinical characteristics	Serum total IgE (IU/ml)	*Acacia* pollen extract		rAca f 1-specific IgE
				Skin prick test[1]	Specific IgE[2]	
1.	26/M	A, R, L	360	10	1.93	1.25
2.	37/F	A, R	252	8	1.72	0.78
3.	30/F	A, R	178	8	1.86	1.00
4.	20/M	A, L	164	6	0.96	0.67
5.	24/M	A, R, L	291	9	0.86	0.76
6.	22/F	A, R	135	7	1.50	0.93
7.	35/F	A, R	158	6	0.83	0.95
8.	34/F	A	132	9	1.80	1.10
9.	30/M	A, R	164	8	1.32	1.12
10.	31/F	A, L	174	6	0.70	0.71
11.	22/F	A, R	224	8	1.58	0.94

M – male; F – female; A – allergic rhinitis; L – lung symptoms (breathlessness, tight chest, cough, wheeze); R – rhinoconjunctivitis
[1]The mean wheal areas are displayed in mm². Histamine diphosphate (10 mg/ml) – positive control, glycerine – negative control.
[2]Determined in specific ELISA as OD (optical density) at 450 nm

Results

Patients and SPTs

Twenty-five patients – 11 males and 14 females (mean age: 28.56 ±5.93 years; age range 19-40 years) – were included in present study. All patients suffered from respiratory allergies and seasonal rhinitis during pollination season of *Acacia*. The patients were all positive by SPT with *Acacia* pollen extracts (Table 1). A serum pool of five non-allergic subjects who showed negative SPT responses and no specific IgE against *Acacia* pollen extract were considered as negative control.

Total and specific IgE levels

The mean total IgE of patients' sera was determined as 256.63 IU/ml. In Aca f 1 reactive patients, the mean of total IgE was 202.90 IU/ml (Table 1). Serum from 25 allergic patients were assessed for specific IgE binding to proteins from *Acacia* pollen extract. All of these patients had significantly elevated specific IgE levels to the extract of *Acacia* pollen (mean: $OD_{450,}$ 1.15 ±0.46; range: 0.70-2.3). The mean OD_{450} for specific IgE in rAca f 1 reactive patients was 1.36 ±0.45; range: 0.70-1.93) (Table 1).

Analysis of nucleotide and deduced amino acid sequences of Aca f 1

The sequence analysis of Aca f 1 allergen showed an open reading frame of 453-bp coding 150 amino acid residues with a predicted molecular mass of 16.525 kDa and a calculated isoelectric point (pI) of 4.62. The obtained nucleotide sequence was submitted to the NCBI GenBank (Accession Number: KR870435).

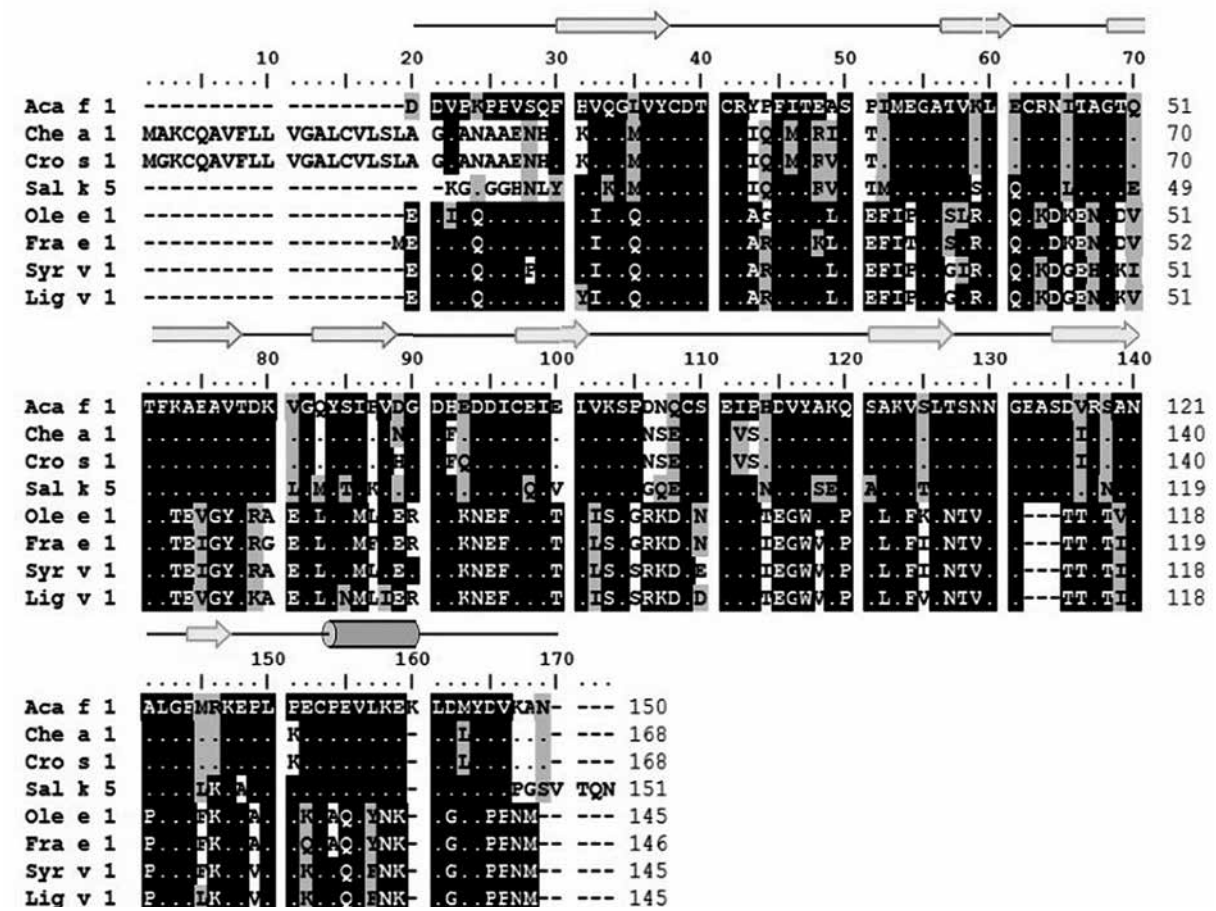

Fig. 1. Comparison of the *Acacia farnesiana* Ole e 1-like protein (Aca f 1) amino acid sequence with allergenic Ole e 1-like protein from other plants: *Chenopodium album* (Che a 1, G8LGR0.1), *Crocus sativus* (Cro s 1, XP004143635.1), *Salsola kali* (Sal k 5, ADK22842.1), *Olea europaea* (Ole e 1, P19963.2), *Fraxinus excelsior* (Fra e 1, AAQ83588.1), *Syringa vulgaris* (Syr v 1, S43243), and *Ligustrum vulgare* (Lig v 1, O82015.2). The amino acid sequence identity and the similarity of Aca f 1 (KR870435) to other members of the Ole e 1-like family are indicated in Table 2. The top line indicates the location of secondary structures created by PSIPRED protein sequence analysis (http://bioinf.cs.ucl.ac.uk/psipred/). Cylinder, arrows, and black line correspond to α-helices, β-strands, and coil structure, respectively

Table 2. Percentage of similarity and identity between Aca f 1 and selected allergenic Ole e 1-like proteins

Allergens	GenBank Accession No.	Aca f 1	
		% Similarity	% Identity
Che a 1	G8LGR0.1	89	82
Cro s 1	AAX93750.1	88	82
Sal k 5	ADK22842.1	90	77
Ole e 1	P19963.2	63	48
Fra e 1	AAQ83588.1	62	48
Syr v 1	S43243	63	46
Lig v 1	O82015.2	62	46

Che a 1 – Chenopodium album; Cro s 1 – Crocus sativus; Sal k 5 – Salsola kali; Ole e 1 – Olea europaea; Fra e 1 – Fraxinus excelsior; Syr v 1 – Syringa vulgaris; Lig v 1 – Ligustrum vulgare

To evaluate sequence homology between Aca f 1 and the known allergenic Ole e 1-like protein in the protein database, the deduced amino acid sequences of these allergenic proteins were compared (Fig. 1). A high degree of sequence identity (82%) was detected between Aca f 1 and Che a 1 and Cro s 1 (allergen from *Crocus sativus* pollen) (Table 2).

SDS-PAGE and IgE-binding components of *Acacia* pollen extract

The SDS-PAGE separation of the pollen extract showed several protein bands in the *Acacia* pollen extract with molecular weights ranging from approximately 10 to 85 kDa (Fig. 2). IgE-binding reactivity of the separated protein bands from the electrophoresis of the *Acacia* pollen extract was assessed by conducting immunoblotting experiments. The results indicated several IgE-reactive bands ranging from around 12 to 85 kDa.

Expression and purification of Aca f 1

A pET-21b (+)/Aca f 1 clone was created and confirmed by digestion with *Not*I and *Xho*I restriction enzymes. This recombinant plasmid was expressed in *E. coli* strain BL21 (DE3) pLysS as a fusion protein with His_6-tag in the C-terminus.

The rAca f 1 was present in a soluble form in the supernatant, where it was further purified by Ni^{2+} affinity chromatography to yield purified protein. The purified rAca f 1 was quantified using Bradford's protein assay, which indicated that nearly 14 mg of recombinant protein had been purified from one litre of the bacterial expression medium. SDS-PAGE revealed that the apparent molecular weight of the fusion protein was about 17.5 kDa (Fig. 3). The allergenic Ole e 1-like protein from *Acacia* pollen, as a new allergen, was designated Aca f 1 by the WHO/IUIS Allergen Nomenclature Subcommittee.

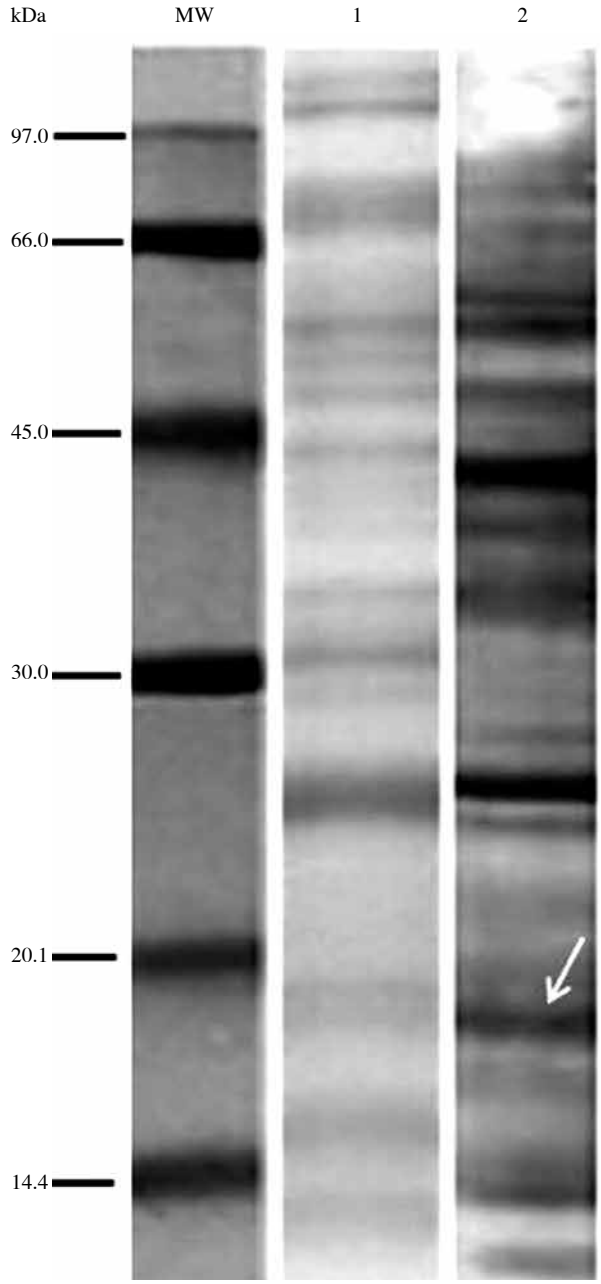

Fig. 2. SDS-PAGE and immunoblotting of *Acacia farnesiana* pollen extract. Lane MW: molecular weight marker (GE Healthcare, Little Chalfont, UK); lane 1: Coomassie Brilliant Blue stained SDS–PAGE of the crude extract of *Acacia pollen* (12.5% acrylamide gel); lane 2: Immunoblotting of *Acacia pollen* extract. The strip was first blotted with *Acacia pollen* extract and subsequently incubated with pooled sera of *Acacia* allergic patients ($n = 5$) and detected for IgE reactive protein bands. Natural Aca f 1 is shown by an arrow

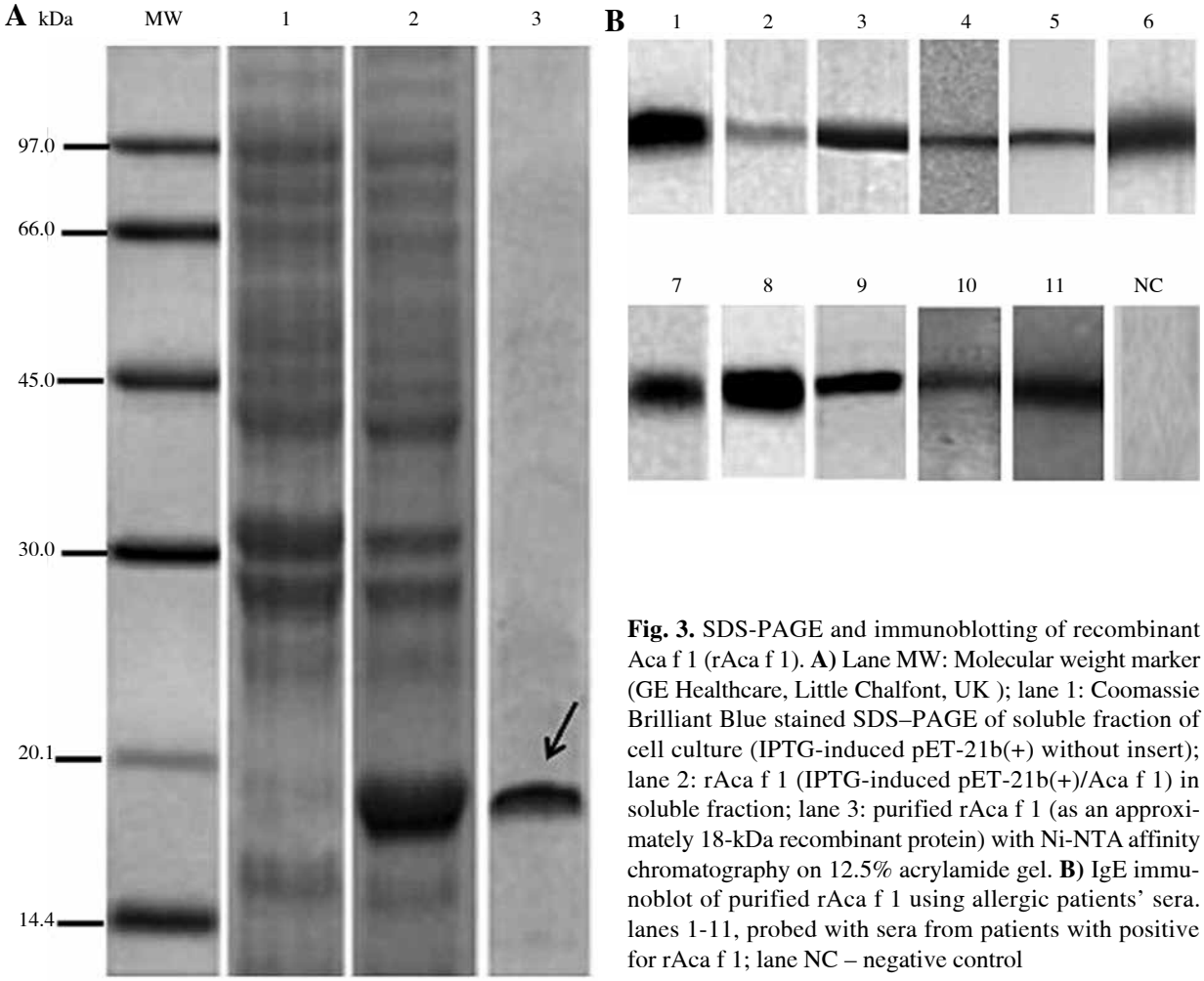

Fig. 3. SDS-PAGE and immunoblotting of recombinant Aca f 1 (rAca f 1). **A)** Lane MW: Molecular weight marker (GE Healthcare, Little Chalfont, UK); lane 1: Coomassie Brilliant Blue stained SDS–PAGE of soluble fraction of cell culture (IPTG-induced pET-21b(+) without insert); lane 2: rAca f 1 (IPTG-induced pET-21b(+)/Aca f 1) in soluble fraction; lane 3: purified rAca f 1 (as an approximately 18-kDa recombinant protein) with Ni-NTA affinity chromatography on 12.5% acrylamide gel. **B)** IgE immunoblot of purified rAca f 1 using allergic patients' sera. lanes 1-11, probed with sera from patients with positive for rAca f 1; lane NC – negative control

Analysis of IgE-binding potential of rAca f 1

The levels of specific IgE to the purified rAca f 1 were determined using 25 individual patients' sera. Eleven patients (44%) had significant specific IgE levels to rAca f 1 (Table 1). Serum samples from patients allergic to *Acacia* pollen were subsequently tested for IgE reactivity to rAca f 1 in immunoblotting experiments. The results showed that the recombinant form of Aca f 1 was reactive with 11 individuals' sera (Fig. 3). These results were consistent with those obtained from specific IgE ELISA (Table 1).

In vitro inhibition assays

ELISA inhibition experiments were performed to investigate the IgE-binding potential of the purified rAca f 1 compared to its natural form in *Acacia* pollen crude extract. ELISA inhibition results revealed a dose-dependent inhibition of the IgE directed to rAca f 1 in patients' sera positive to *Acacia* pollen extract. Pre-incubation of pooled serum with 1 mg/ml of rAca f 1 and *Acacia* pollen extract revealed significant inhibition (92% and 85%, respectively) of IgE binding to rAca f 1 in microplate wells (Fig. 4).

Immunoblot inhibition assays showed that pre-incubation of serum samples with rAca f 1 nearly completely inhibited the IgE binding to a protein band with an apparent molecular weight of 17 kDa (Fig. 5, line 3). Altogether, *in vitro* inhibition assays revealed a similar IgE reactivity for rAca f 1 and its natural counterpart in *Acacia* pollen extract. In addition, results indicated that pre-incubation of serum samples with native crude extract of *Acacia* pollen inhibited IgE binding to natural Aca f 1 in *Acacia* pollen extract and other reactive proteins (Fig. 5, line 2). However, pre-incubation of the pooled serum with BSA did not affect the IgE-reactivity to rAca f 1 (Fig. 5, line 1).

Discussion

In recent years, the pollen of *Acacia* species such as *A. farnesiana* has been recognised as a cause of respiratory allergy in various parts of the Southern hemisphere [1, 4, 6, 7, 27-31]. Up to now, only one allergen (Aca f 2) of *Acacia*

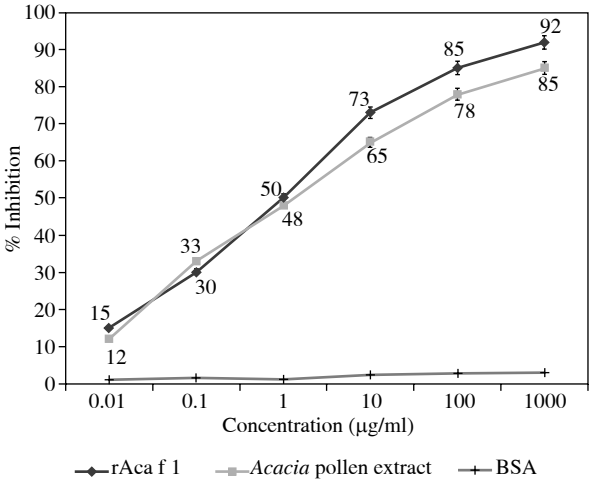

Fig. 4. ELISA inhibition with *Acacia farnesiana* pollen extract and rAca f 1. Inhibition of IgE-binding to rAca f 1 by ELISA using *Acacia* pollen extract and rAca f 1. Control experiments were performed with BSA

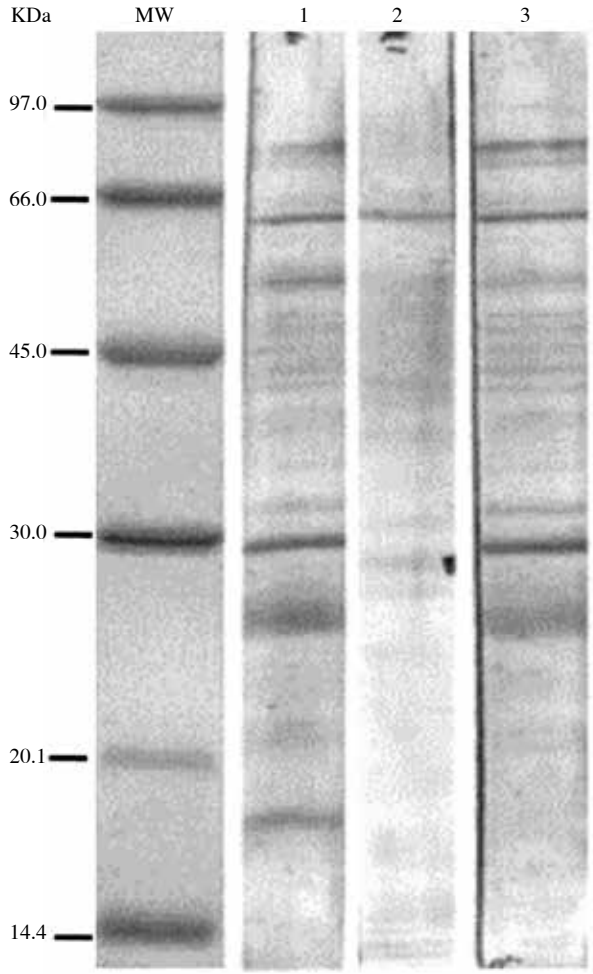

Fig. 5. Immunoblotting inhibition assays. Lane MW: molecular weight marker (GE Healthcare, UK); lane 1: *Acacia* protein strip incubated with pooled serum without inhibitor (negative control); lane 2: *Acacia* protein strip incubated with pooled serum containing 85 µg of *Acacia* pollen extract as inhibitor (positive control); lane 3: *Acacia* protein strip incubated with pooled serum containing 30 µg purified rAca f 1, as an inhibitor

pollen has ever been identified as a member of the profilin family [8]. In this study a cDNA encoding another major allergen from *Acacia* pollen has been cloned, expressed, and identified as a member of the Ole e 1-like protein family, and its importance has been analysed with a population of patients living Ahvaz city, Southwest Iran, a tropical region in which *Acacia* pollen induces nearly 48% of the total sensitisation of patients with allergic rhinitis [4]. This allergenic member of the Ole e 1-like protein family of *A. farnesiana* was named Aca f 1 in accordance with the International Union of Immunological Societies (IUIS) Allergen Nomenclature Subcommittee (http://www.allergen.org/). The IgE-reactivity to some allergenic members of Ole e 1-like protein family has been demonstrated between 30% and 77% in allergic patients in different regions where it was considered as a major allergen in pollen of the allergenic plants such as *O. europaea* (Ole e 1), *F. excelsior* (Fra e 1), *C. album* (Che a 1), *S. kali* (Sal k 5), and *Ligustrum vulgare* (Lig v 1) [15, 17, 18, 23, 24].

The open reading frame contains 453 bases encoding a mature Aca f 1 polypeptide of 150 amino acids, with six cysteine residues. Encoded within this open reading frame is a 16.5 kDa protein that exhibits molecular characteristics similar to known members of the plant Ole e 1-like protein family (ranging from 16 to 19 kDa) [14, 17, 24]. Moreover, Aca f 1, like Che a 1, Cro s 1, and Sal k 5 has a conserved sequence for potential *N*-glycosylation in the same position of the polypeptide chain (Asn-Ile/Leu-Thr-Ala), which is actually occupied by a glycan in these proteins.

The results of immunoblotting assay on *Acacia* pollen extract using pooled sera from patients also revealed an IgE-binding protein band with an estimated MW of 17 kDa (Fig. 1).

The IgE-binding capacity of the purified rAca f 1 to sera from *A. farnesiana* allergic patients was evaluated using specific ELISA and immunoblotting assays in order to confirm that rAca f 1 was correctly folded and bound to IgE as the natural counterpart in *Acacia* extract. The immunoblot analysis with individual patients' sera demonstrated diverse IgE reactivity with six proteins of 15, 17, 28, 39, 45, and 50 kDa as major IgE binding components. The results of immunoblotting assays for natural Aca f 1 with an apparent molecular weight of 17 kDa were consistent with those obtained from rAca f 1. A nearly complete inhibition of IgE-binding to natural Aca f 1 was also obtained after pre-incubation of pooled sera with purified rAca f 1.

It seems that rAca f 1 is comprised of IgE-epitopes similar to those of its natural counterpart.

Cross-reactivity among *Acacia* pollen components with other allergenic plants has been described [1, 11, 12]. The importance of the *Acacia* and the most allergenic members of the *Fabaceae* (*Prosopis juliflora*) and the *Amaranthaceae* (*Salsola kali*, *Chenopodium album*) families' pollens have been described as main causes of respiratory allergy [3-5, 7]. This study was performed to elucidate the amino acid sequence homology of Ole e 1-like proteins to prevalent allergenic regional plants. The results of amino acid sequence identity analyses revealed that Aca f 1 has a significant degree of identity with selected members of the allergenic Ole e 1-like protein family from the most common allergenic regional plants, particularly *C. album* (Che a 1), *Crocus sativus* (Cro s 1), and *S. kali* (Sal k 5) (82%, 82% and 77%, respectively). Identification of the Aca f 1 sequence will allow further studies on the basis of *in vitro* assays to evaluate the molecular basis of cross-reactivity between these important allergenic pollens.

In conclusion, Aca f 1, with a detectably specific IgE in 44% of *Acacia* allergic patients and the second allergen from the *A. farnesiana* pollen was identified as a member of the Ole e 1-like protein family. Furthermore, the results indicate that *E. coli* is a relevant expression system for the production of rAca f 1 with immunoreactivity similar to that of the allergen's natural form. Analysis of the amino acid sequences of Aca f 1 and several allergenic members of the Ole e 1-like protein family from other plants also showed a possibility of cross-reactivity among plants belonging to unrelated families, which may be predicted by the degree of amino acid sequence identity of potential conformational epitopes. However, this hypothesis needs to be confirmed in further studies where cross-reactivity among these allergens are evaluated using in vitro assays.

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The authors declare no conflict of interest.

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