

Sal k 4, a New Allergen of *Salsola kali*, Is Profilin: A Predictive Value of Conserved Conformational Regions in Cross-Reactivity with Other Plant-Derived Profilins

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The aim of this study was to investigate a new allergen of *Salsola kali*, Sal k 4, and to investigate the predictive value of the conserved conformational regions in cross-reactivity with other plant-derived profilins. The Sal k 4-coding sequence was cloned, expressed, and purified by one-step Ni²⁺ affinity chromatography to recover high-purity target protein. We assessed cross-reactivity and predicted conserved conformational regions among rSal k 4 and other plant-derived profilins. Immunodetection and inhibition assays using 30 individual sera from *S. kali* allergic patients indicated that purified rSal k 4 might be the same as that in the crude extract. The results of inhibition assays among rSal k 4 and other plant-derived profilins were in accordance with the homology of the predicted conserved conformational regions. Amino acid sequence homology analysis showed that a high degree of IgE cross-reactivity among plant-derived profilins might depend on the predicted conserved conformational regions.

Key words: Sal k 4; *Salsola kali*; profilin; cross-reactivity

Allergy to the pollen of *Salsola kali* (*S. kali*), or (*Russian thistle*), is common in the western United States, the Mediterranean area and Asia.^{1–4} This plant and various other genera of the Chenopodiaceae family are abundant throughout desert and semi-desert regions of Iran, Saudi Arabia, and Kuwait.^{1,3,5,6} Moreover, sensitization to *S. kali* pollen is an important cause of allergic respiratory symptoms in these regions, where the frequency of sensitization ranges from 53% to 76.7%.^{1,3–7} The Sal k 1, a 43 kDa allergen of *S. kali* pollen was recently described as a pectin methylesterase, and is considered a major allergen of *S. kali* pollen based on the use of sera from Spanish *Salsola* pollen-allergic individuals.^{8,9}

Profilins are 12- to 15-kDa monomeric proteins belonging to a ubiquitous family of actin-binding proteins. They are prominent allergens in the pollens of trees, grasses, and weeds, and many fruits and vegetables,^{10,11} but these allergens are usually under-

represented in natural extracts used for diagnostic, therapeutic, and experimental purposes.

In this study, for the first time, the recombinant profilin of the *S. kali* pollen was produced in *Escherichia coli* at a relatively high level of expression. After purification, the allergenic reactivity of the recombinant protein and its cross-reactivity with other plant-derived profilins were examined using sera from *S. kali* allergic patients. *S. kali* pollen profilin, a new allergen, was designated Sal k 4 by the WHO/IUIS Allergen Nomenclature Subcommittee.

Materials and Methods

Patients, skin prick test (SPT) and recombinant profilins. The study included 30 patients suffering from allergy to *S. kali* pollen, 18 women and 12 men who had seasonal rhinitis without asthma. All the subjects had positive SPTs to *S. kali* pollen extract. Four other healthy subjects who showed negative SPT responses and no specific IgE to *S. kali* pollen extract used as negative control. Case histories in respect to *S. kali* allergy are summarized in Table 1.

Two g of *Salsola kali* defatted pollen at a purity of more than 95%, purchased from Greer Laboratories (Lenoir, NC), and were extracted as described previously.^{5,6} The protein content of the extract was then measured by the Bradford method.¹² SPT with *S. kali* pollen extract was performed as previously described.⁵ With informed written consent from each patient, the study was approved by the Human Ethics Committee of Mashhad University of Medical Sciences (MUMS). Recombinant profilins were from *Crocus sativus* (rCro s 2) expressed in *E. coli* and purified by metal precipitation.¹³ Recombinant profilins from *Amaranthus retroflexus* (rAma r 2), *Chenopodium album* (rChe a 2), and *Cucumis melo* (rCuc m 2) were expressed in *E. coli* and purified by metal affinity chromatography (Ni-NTA) (unpublished data).

Amplification of Sal k 4 cDNA by PCR and determination of nucleotide sequence. The Sal k 4 coding region was amplified using degenerate primers designated on the basis of amino acid sequence comparisons of various plant profilins. A PCR product 399 bp in length was cloned into the pTZ57R/T vector from the Ins TAclone™ PCR cloning kit (Fermentas, Vilnius, Lithuania). The resulting plasmids were employed to transform TOP10 *E. coli* cells, and recombinant plasmids were then subjected to sequencing (SeqLab, Göttingen, Germany).

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Abbreviations: ELISA, enzyme-linked immunosorbent assay; IPTG, isopropyl β-D-thiogalactopyranoside; IUIS, International Union of Immunological Societies; NCBI, National Center of Biotechnology Information; PCR, polymerase chain reaction; PVDF, polyvinylidene fluoride; SPT, skin-prick test

Table 1. Clinical Characteristics, SPT Responses, and IgE-Reactivity of Patients Allergic to *S. kali* Pollen

Patients	Age (years)/Sex	Clinical history [†]	<i>S. kali</i> extract		Recombinant Sal k 4	
			Skin test [‡]	Specific IgE [†]	Skin test	Specific IgE
1	18/M	R,RC	25	0.93	15	0.80
2	9/F	R	30	1.13	20	0.95
3	18/F	R	23	0.84	10	0.66
4	21/F	R	26	0.89	0	0.18
5	29/M	R, RC	20	0.74	0	0.20
6	22/F	R	27	1.08	16	0.85
7	28/F	R, RC	26	1.08	0	0.23
8	36/F	R, RC	20	0.69	0	0.25
9	20/F	R, RC	27	1.07	12	0.44
10	21/F	R	25	0.89	0	0.21
11	22/F	R, RC	28	0.98	15	0.78
12	13/F	R	25	0.84	0	0.23
13	14/F	R	20	0.71	13	0.65
14	23/F	R	24	0.92	14	0.73
15	39/M	R	23	0.79	0	0.14
16	38/F	R	30	1.05	17	0.75
17	25/M	R, RC	23	0.78	14	0.70
18	27/F	R, RC	28	1.02	0	0.28
19	29/M	R	18	0.68	0	0.16
20	20/M	R, RC	22	0.83	15	0.80
21	20/M	R	19	0.79	0	0.18
22	27/F	R	25	0.94	0	0.24
23	25/M	R	27	0.89	0	0.12
24	36/F	R, RC	31	1.12	17	0.79
25	8/M	R	26	0.84	0	0.26
26	27/M	R	15	0.72	0	0.15
27	20/M	R, RC	24	0.85	12	0.63
28	16/M	R	23	0.79	0	0.17
29	38/F	R, RC	20	0.89	14	0.70
30	13/F	R, RC	21	0.76	0	0.25
Controls						
31	34/F	—	0	0.10	0	0.06
32	24/F	—	0	0.10	0	0.08
33	29/M	—	0	0.09	0	0.07
34	35/F	—	0	0.17	0	0.06

[†]AR, Allergic rhinitis; RC, rhinconjunctivitis.[‡]The mean wheal areas are displayed in mm². Histamine diphosphate (10 mg/ml)-positive control; glycerin-negative control.[†]Determined by specific ELISA as OD (optical density) at 450 nm.

Construction of prokaryotic expression plasmid carrying Sal k 4 gene. Based on the sequence obtained, specific primers for Sal k 4 were designed. These contained overhangs with *Not* I and *Xho* I restriction sites for direct cloning into expression plasmid pET-32b(+) (Novagen, Gibbstown, NJ) as follows: sense primer (5'-ACCGCGGCCGCAATGTCCTGGCAGACGTATGTTGA-3', *Not* I restriction site underlined), and anti-sense primer (5'-CCCTCGAGCATGCCTTGTTTCGACCAGATAGT-3', *Xho* I restriction site underlined).

The resulting 399-bp amplified product was digested with *Not* I and the *Xho* I restriction enzymes according to the manufacturer's protocol (Fermentas). The purified digested PCR product was ligated into the digested pET-32b(+) plasmid with the same enzymes. The pET-32b(+)/Sal k 4 expression vector provided a sequence coding for a thioredoxin (TrxA) partner and two hexahistidyl (6 × His) affinity tags at the 5' and 3' ends of the Sal k 4 coding sequence. Correct constructs were transformed into competent *E. coli* BL21 (DE3) cells (Novagen).

Expression and purification of recombinant Sal k 4 (rSal k 4). A fresh clone of recombinant plasmid pET-32b(+)/Sal k 4 was inoculated into 2 ml of LB medium containing 100 µg/ml of ampicillin and this was incubated at 37 °C. Expression of the recombinant protein was induced by the addition of isopropyl β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.2 mM. After induction, in order to improve the solubility of rSal k 4, the cultures were incubated at 18 °C and the cells were allowed to grow for a period of 12 h. Subsequently, the cells were harvested by centrifugation (3,000 g, 15 min, 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 subjected to 3 freeze-thaw cycles with liquid nitrogen. Purification of

rSal k 4 was done with Ni-NTA agarose (Invitrogen) from the soluble phase of lysate, following, the manufacturer's instructions.

IgE-Immunoblotting and IgE-immunoblotting inhibition. The natural extract of *S. kali* pollen and purified rSal k 4 were electrophoresed on 12.5% SDS-PAGE and then electrotransferred onto polyvinylidene difluoride (PVDF) membranes (Immobilon-P, Millipore, Billerica, MA), as described elsewhere.⁵⁾

To study cross-inhibition between natural *S. kali* pollen extract and rSal k 4, a mixture of 100 µl of pooled serum (1:5 v/v) was incubated with natural *S. kali* pollen extract (20 µg/ml, as inhibitor), rSal k 4 (10 µg/ml, as inhibitor), or BSA (as negative control) for 3 h at 37 °C with shaking. Preincubated sera were used to assess the reactivity of a PVDF membrane blotted with natural *S. kali* extract and rSal k 4, as described above.

Indirect enzyme-linked immunosorbent (ELISA) and inhibition assays. To evaluate the specific IgE reactivity of the *S. kali* pollen extract, all 30 sera were assessed by ELISA, as previously described.¹⁴⁾ Four control subjects who showed negative SPT responses to *S. kali* pollen extract (Table 1, sample numbers 31–34) were also used as negative controls. An optical density (OD₄₅₀) greater than 4 times the median values of the negative controls was considered to be positive.

To determine the dose-dependence of IgE reactivity to Sal k 4 by other plant profilins, including rAma r 2, rChe a 2, rCuc m 2, and rCro s 2, ELISA inhibition assays were performed as described above, except that a pooled serum (1:2 v/v) from *S. kali* allergic patients (Nos. 1, 2, 3, 11, 14, and 16) was preincubated for 1 h at room temperature with 100, 10, 1, 0.1, or 0.01 µg of the purified specific

	10	20	30	40	50	60	70	80	
Sal k 4	MSWQTYVDDHLMCEIEGTNNHLTAAAILGVDGSVVAQSANFPQFKPD	ISAVVKEFDEAGTLAPTGLHLG	GTKYMIQGE						
Ama r 2	A.....T.....G.....L.....D.....A.I..ED..P.....								
Hev b 8	D.D.--HR.....I.H.....SS.....S.VA..M.D..P.S.....								
Che a 2	FP..E.G.....V.L.....ST..L.QE.VK.ICN..VPN.....F..E.....								
Cuc m 2	V..E.....S..I.Q.....Q..L..E.VAGI..GD.ADP.....YI.....								
Cro s 2	E.....DMD.--HV..S.....H.....G..EL..A..T..ILND.N.P.S.....								
	90	100	110	120	130				
Sal k 4	AGQVIRGKKGPGGICVKKTGQALIFGIYDEPVTTPQCNMIVERLGDYLVEQGM	133	%Identity	%Similarity					
Ama r 2	P.A.....A.....VM.....I...Y	133	87	93					
Hev b 8	P.A.....S..T.....I.....L.....LD..L	131	82	91					
Che a 2	P.A.....V.I.....N.....V..K.....	133	77	86					
Cuc m 2	P.A.....AT.....M..VI.....M.....ID..L	131	75	87					
Cro s 2	P.V.....S..VTI.....NM.....L...M.....LV.....I...Y	131	68	87					

Fig. 1. Comparison of the Sal k 4 Amino Acid Sequence with Allergenic Profilins from Other Plants.

The amino acid sequence identity and the similarity of Sal k 4 (GQ145223) to other members of the profilin family are indicated at the ends of the amino acid sequences. *Amaranthus retroflexus* (Ama r 2, ACP43298), *Hevea brasiliensis* (Hev b 8, CAA75312), *Chenopodium album* (Che a 2, ACR77509), *Cucumis melo* (Cuc m 2, AAW69549), and *Crocus sativus* (Cro s 2, AAW81034). The highly conserved residues in plant-derived profilins involved in biological and structural functions (Poly-L-proline binding, fold conservation, actin binding site, and stabilized turn) are shaded in gray.

Table 2. Comparison of Plant-Derived Profilins with Sal k 4 Based on Predicted Conformational Epitopes[†]

IgE-Binding epitope sequences	Plant-derived profilins (% identity)					Mean of identity (%) in each epitopes
	Ama r 2	Cuc m 2	Hev b 8	Che a 2	Cro s 2	
1 2SWQTYVDDH ₁₀ Q ₃₇ Y ₁₂₇ Q ₁₃₁ GM ₁₃₃	85.7	78.5	92.8	100	85.7	88.54
2 8DDH ₁₀ 13CEIEGNHLT ₂₃ 39AN ₄₀ 109DEPVT ₁₁₃	94.7	84.2	63.1	73.6	57.8	74.68
3 Q4D ₈ 21HLT ₂₃ V ₃₄ 37QSANFPQ ₄₃ G ₇₁ D ₁₀₉	93.3	93.3	80	80	73.3	83.98
4 32GSV ₃₄ 38QSANFPQFKPD ₄₇ H ₆₈ 71GT ₇₂	94.1	76.4	82.3	64.7	64.7	76.44
5 30VDGSV ₃₄ 42PQFKPD ₄₇ 50SAVVK ₅₄ T ₆₅ H ₆₈ G ₇₁	78.9	57.8	78.9	52.6	57.8	65.2
6 D ₄₇ 50SA ₅₁ K ₅₄ 60GTLAPT ₆₅ H ₆₈ 71GTKYMV ₇₆	88.2	70.5	88.2	64.7	64.7	75.26
7 59AG ₆₀ A ₆₃ 75MVIQGEAGQ ₈₃ 86RGK ₈₈ P ₁₁₄ N ₁₁₈ E ₁₂₂	88.8	88.8	83.3	83.3	83.3	85.5
8 53VK ₅₄ 57DEAGT ₆₁ 76VIQGEAGQ ₈₃ R ₈₆ K ₉₈ E ₁₂₂	83.3	72.2	72.2	66.6	61.1	71.08
9 17GTN [‡] NHLT ₂₃ 88KKG ₉₁ 109DEPVT ₁₁₄	93.3	93.3	73.3	86.6	73.3	83.96
10 H ₁₀ 13CEIEGTN [‡] N ₂₀ R ₈₆ 113TPG ₁₁₆ 118NM ₁₁₉ 122ER ₁₂₃ 126GY ₁₂₇	100	100	76.4	76.4	64.7	83.5
11 Y ₆ 98KTGQ ₁₀₁ 122ER ₁₂₃ 126DYLVEQGM ₁₃₃	86.6	73.3	80	86.6	66.6	78.62
Mean of identity (%) in each plant-derived profilins	89.7	80.7	79.1	75.9	68.4	

[†]Adapted from reference 17. Residue numbering corresponds to a position in *S. kali*.

[‡]These residues (in italics) were not found in Hev b 8, Cuc m 2, and Cro s 2.

recombinant proteins as inhibitor or with BSA as negative control. Serum without rSal k 4 served as a sample without an inhibitor. Assays were performed in duplicate. The percentage of inhibition was calculated using the following formula: (OD of sample without inhibitor – OD of sample with inhibitor/OD of sample without inhibitor) × 100.

Results

Cloning, sequence analysis, and homology modeling of Sal k 4

Sequence analysis of Sal k 4 showed an open reading frame of 399 bp coding for 133 amino acid residues with a predicted molecular mass of 14.274 kDa and a calculated pI of 4.66. The sequence obtained was submitted to the NCBI GenBank (accession no. GQ145223) (Fig. 1).

A comparison of the deduced amino acid sequence of the *S. kali* pollen profilin with other allergenic plant-derived profilins in the protein database was done (Fig. 1). A high level of sequence identity (87%) was detected between Sal k 4 and Ama r 2. Moreover, the most conserved residues showed involvement in specific biological functions and structural roles (Fig. 1).

The amino acid sequences of selected profilins were evaluated in 11 predicted conformational epitopes, as recently reported.¹⁵⁾ Based on these epitopes, the highest

level of identity (89.7%) was found between Sal k 4 and Ama r 2 (Table 2). Moreover, in accordance with a previous study, epitopes 1 and 7 contained more conserved residues in selected profilins (88.54% and 85.5% respectively).

A homology model of *S. kali* profilin was generated using coordinates from the profilin of *Arabidopsis thaliana* (PDB no. 3nua) by the Internet server Swiss Model (<http://swissmodel.expasy.org/SWISS-MODEL.html>), as previously described.¹⁴⁾ The profilin of *Arabidopsis thaliana* had 75% sequence identity with the *S. kali* profilin (Fig. 2).

Expression and purification of rSal k 4

A pET-32b(+)/Sal k 4 clone was constructed and confirmed by digestion with *Not* I and *Xho* I restriction enzymes. This recombinant plasmid, containing TrxA to increase solubility, and two his-tags for Ni-NTA purification, was expressed in *E. coli* strain BL21 (DE3)pLysS as an approximately 34-kDa recombinant protein (TrxA-rSal k 4). The purified rSal k 4 was quantified by Bradford's protein assay, which showed that approximately 2.2 mg of recombinant protein had been purified from 1 liter of the bacterial expression medium.

IgE-Immunoblot and immunoblot inhibition of rSal k 4

Analysis of both natural forms of profilin (Sal k 4) in the *S. kali* pollen extract and purified recombinant protein revealed that they were reactive with 14 (46.6%) individual sera (Fig. 3B and C), lanes 1, 2, 3, 6, 9, 11, 13, 14, 16, 17, 20, 24, 27, and 29).

Inhibition experiments demonstrated similar IgE reactivity for purified rSal k 4 and its natural counterpart in the *S. kali* pollen extract. The results indicated that pre-incubation of serum samples with the crude extract of *S. kali* pollen (Fig. 4, lane 2) or rSal k 4 (Fig. 4, lane 3) completely inhibited IgE binding to natural Sal k 4. However, preincubation of the pooled serum with PBS-BSA had no influence on its IgE-reactivity to Sal k 4 (Fig. 4, Lane 1).

Specific IgE ELISA and inhibition assay

Specific IgE values against *S. kali* pollen extract and purified rSal k 4 were determined with 30 individual

patients' sera (Table 1). All of these patients had significantly elevated specific IgE levels to *S. kali* pollen extract. Fourteen sera (14/30) gave OD values of 450 nm > 0.3 (4 times greater than the median values for the negative controls), and thus were considered capable of binding to purified rSal k 4 (Table 1). These results are consistent with those obtained in the IgE immunoblotting assays using the recombinant protein.

To investigate cross-reactivity among rSal k 4 and other weed and fruit profilins, ELISA inhibition with recombinant profilins (rAma r 2, rChe a 2, rCuc m 2, and rCro s 2) was done (Fig. 5). Almost complete inhibition was attained with 100 µg/ml of rSal k 4 as positive

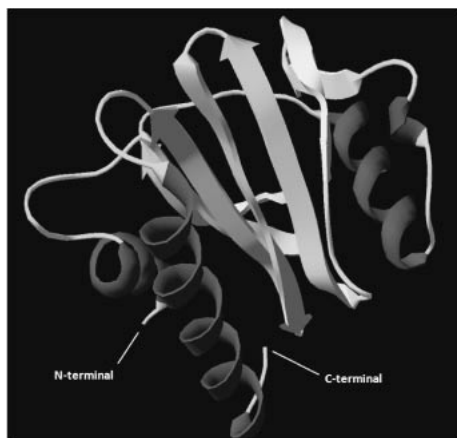


Fig. 2. Homology Model of *S. kali* Profilin.

Ribbon representation of the structure of Sal k 4 modeled according to the three-dimensional structure of the profilin of *Arabidopsis thaliana* (PDB no. 3nula).

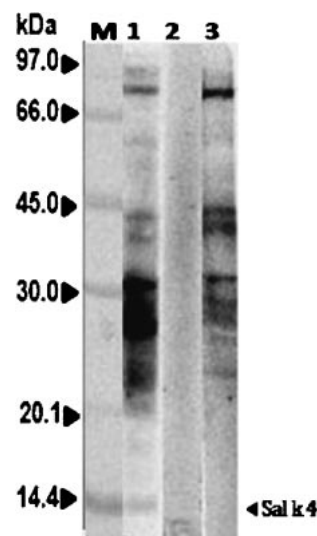


Fig. 4. IgE-Immunoblotting Inhibition Analysis.

Pollen extract of *S. kali* was separated by SDS-PAGE and blotted onto PVDF. IgE-Binding of a pooled serum, including six *S. kali* allergic patients (nos. 1, 2, 3, 11, 14, and 16) after preincubation with PBS (lane 1), *S. kali* extract (20 µg/ml) (lane 2), and purified rSal k 4 (10 µg/ml) (lane 3). The IgE reactivity of allergic sera with natural Sal k 4 was inhibited after incubation with rSal k 4; lane M, molecular weight marker (Amersham).

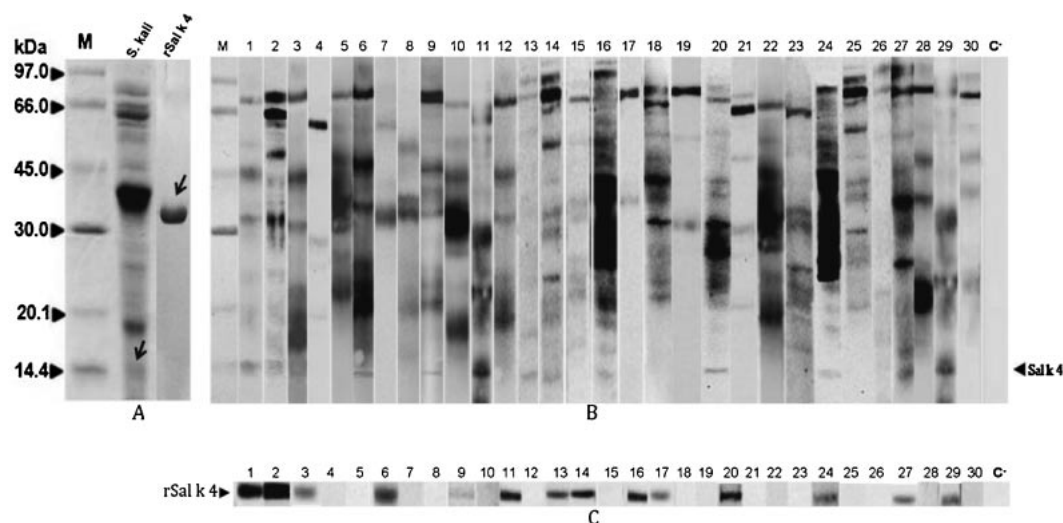


Fig. 3. SDS-PAGE and Immunoreactivity of *S. kali* Pollen Extract and Recombinant Sal k 4.

A, Coomassie Brilliant Blue stained SDS-PAGE of the crude extract of *S. kali* pollen and purified rSal k 4 (as an approximately 34-kDa recombinant protein) on 12.5% acrylamide gel. Lane M, molecular weight marker (Amersham, Buckinghamshire, UK); B, IgE immunoblot of *S. kali* pollen extract using allergic patients' sera; lane M, molecular weight marker (Amersham); lanes 1–30, probed with individual patients' sera; lane C⁻, negative control; C, IgE-immunoblotting analysis of rSal k 4. Lanes 1–30, blotted rSal k 4 was probed with individual sera of *S. kali* allergic patients; lane C⁻, negative control.

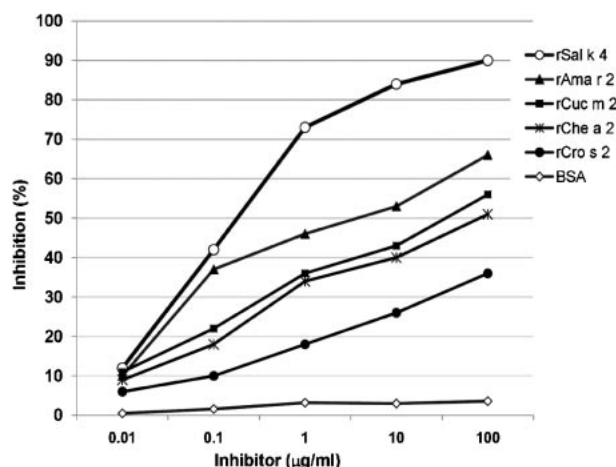


Fig. 5. Inhibition of IgE-Binding to rSal k 4 by ELISA Using Recombinant Profilins from Other Sources: *Amaranthus retroflexus* (rAma r 2), *Chenopodium album* (rChe a 2), *Crocus sativus* (rCro s 2), and *Cucumis melo* (rCuc m 2).

Control experiments were performed with BSA.

control. Pre-incubation of pooled serum with high concentrations (100 µg/ml) of rAma r 2 and rCuc m 2 revealed significant inhibition of IgE binding to rSal k 4 (66% and 56% respectively) (Fig. 5).

Capacity of rSal k 4 to elicit immediate cutaneous reactions

The capacity of rSal k 4 to induce immediate skin reactions was investigated in volunteer *S. kali* allergic patients by *in vivo* SPT (Table 1). Fourteen patients exhibited a positive reaction to rSal k 4. In this way, *in vivo* results confirmed *in vitro* immunoreactivity assays. In the control patients, no skin reactions were elicited by application of purified rSal k 4.

Discussion

Isolated profilins from various sources have indicated high sequence identities and may play roles in IgE-cross reactivity in allergic patients.^{14,16} The purpose of this research was to clone and produce the recombinant profilin from the *S. kali* pollen in *E. coli* in order to obtain the purified rSal k 4.

In this study, profilin of *S. kali* pollen was cloned by a PCR strategy using degenerated primers based on the codons of conserved amino acid sequences from various plant profilins. The open reading frame of Sal k 4 contained 133 bases encoding a 14.3 kDa protein, which correlates with the molecular characteristics of known plant profilins. During the expression of rSal k 4, the temperature of the culture medium was lowered to 18 °C following IPTG induction. As a result, most of the expressed recombinant protein was found in the soluble phase. This confirms that aggregation of protein was fell at a lower temperature, which can be explained by the chaperone-assisted folding pathways in *E. coli*.^{17,18}

The IgE-binding ability of the purified rSal k 4 to sera from *S. kali* allergic patients was evaluated in order to confirm that the recombinant protein was equivalent to its natural counterpart in the crude extract. The results of immunoblotting assays for natural Sal k 4 were consistent with those obtained for recombinant Sal k 4.

Complete inhibition of IgE-binding to natural Sal k 4 was also obtained after preincubation of pooled serum with purified recombinant Sal k 4. Moreover, the results of the following ELISA inhibition experiments were entirely consistent with those obtained in the immunoblotting assays. This study's findings also indicate that, similarly to the results obtained in immunoblot assays with natural Sal k 4 in *S. kali* pollen extract and rSal k 4, 16 patients did not show positive SPTs with rSal k 4. Taking all this, it appears that rSal k 4 is comprised of IgE-epitopes similar to those of its natural counterpart.

In this study, amino acid identity analysis revealed that there were a high degrees of identity among Sal k 4 and other allergenic profilins from other plants in our region, particularly Ama r 2 (*A. retroflexus*), Che a 2 (*C. album*), Cuc m 2 (*C. melo*), and Cro s 2 (*C. sativus*). In spite of this amino acid sequence identity, the results of the ELISA inhibition assays with pooled sera from patients (nos. 1, 2, 3, 11, 14, and 16) showed that inhibition by Cuc m 2 was more intensive than by Che a 2. This was unexpected, because both *S. kali* and *C. album*, but not *C. melo*, belong to the Amaranthaceae family. Concurrently with these results, other studies^{14,15} suggested that profilins from various plant and fruit sources showed differences in their IgE-binding epitopes that might predict cross-reactivity among these proteins, independent of their amino acid sequence homology.^{14,15,19,20} In one of those studies,¹⁵ 11 predicted conformational epitopes were introduced with a binding potential to specific IgE from profilin allergic patients. Amino acid sequence analysis of the 11 predicted conformational epitopes from the selected profilins showed the highest level of identity between the Sal k 4 and Ama r 2 conformational epitope regions (89.7%), and was consistent with the results of ELISA inhibition assays. Furthermore, Radauer *et al.*¹⁵ reported several conserved residues with an IgE-binding potential in 11 predicted conformational epitopes. Those conserved residues were more abundant in predicted epitopes 1 and 7. The present study indicates that compared to the other epitopes, potential conformational epitopes 1 and 7 contained conserved residues, suggesting the essential roles of epitopes 1 and 7 in cross-reactivity among profilins.

In addition to conserved residues with an IgE-binding potential, there are several other residues that differ in profilins from various sources. However, residues playing a crucial role in the structure and biological function of the molecule are significantly conserved among unrelated phylogenetic profilins (Fig. 1).^{21,22}

In conclusion, this study investigated a new allergen from *S. kali* pollen, Sal k 4, with a detectably specific IgE in 46.6% of *S. kali* allergic patients. Moreover, according to their phylogenetic relationships, the cross-reactivity among plant-derived profilins from unrelated families might depend on potential conformational epitopes rather than on the degree of amino acid sequence identity. As an appropriate expression system, *E. coli* BL21 along with the Ni-NTA agarose column, was found to be a tool for high-level production of a pure recombinant allergen suitable for the characterization of allergenic epitopes recognized by T and B cells, and for possible diagnostic and specific therapeutic purposes.

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